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BIOCHEMICAL SOCIETY SYMPOSIA

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NO. 6

THE BIOCHEMISTRY OF FISH

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THE BIOCHEMISTRY OF FISH

A Symposium held at
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INTRODUCTION

By R. A. Morton

Department of Biochemistry, University of Liverpool

Many of us like to regard Biochemistry as an independent science even if, in so doing, we put our own meaning into ' independent ' and ' Biochemistry '. Accepting that as biochemists we have our own way of looking at things, it is beyond dispute that we draw freely upon other sciences for facts and ideas.

The biochemistry of fish is a good illustration of this. A study of the environment of fishes would have to begin with the inorganic solutes of fresh water and sea-water, a subject with very wide implications. The nutrition of fishes leads directly to photosynthesis in phytoplankton and its seasonal variation. The combination of photochemical, tracer and chromatographic techniques has in the hands of the Berkeley and Chicago Schools emphasized that photosynthesis is a central biochemical theme. The dependence of zooplankton populations on phytoplankton fluctuations is the first step in the biological sequence which determines the work of fishermen.

Investigation of the biochemistry of fish requires a brisk traffic to and from other fields of study. The results of this exchange of information and ideas are both stimulating and challenging whether the topic under consideration is reproduction, absorption, metabolism, pigmentation, vision, or a dozen other matters. May I mention a few of the questions within my own experience ?

How are we to interpret the pigmentation of fish eggs and the evidence that some of the carotenoids are ' useful ' but not indispensable ? What are we to make of the extreme variability of squalene in fish-liver oils ? What are its precursors and what is its fate ?

Again, how much remains to be done in the field of the chemistry and biochemistry of the poly-ethenoid long-chain acids of fish oils ? The regulation of growth in fishes is a fascinating problem with important implications for endocrinology.

It is often interesting to speculate how Biochemistry would have developed if some discoveries had been made in a different order. Vitamin A could easily have been first isolated from tunny liver oil or halibut liver oil or visceral oil, in all of which it is a major constituent. Vitamin D_3 might have been isolated from one of the tuna species. Such speculations illustrate a point of some importance. Hitherto a great deal of research has been directed towards isolating active principles responsible for previously defined physiological effects. Isolation techniques have now been so developed that some important substances are likely to be discovered in natural products before the *nature* of their significance. In such cases we shall need the biochemical insight which comes from a wide biological discipline.

From another point of view it is equally important to take a broad view of the *applied* biochemistry of fish. The facts about one sector of the fishing industry are eloquent enough. Fish Bulletin 64 of the California Department of Nature Resources (1946) tells the story of the soupfin shark. From 1930–36 the annual catch was on the average some 270 tons. The discovery in 1937 that soupfin shark livers were very rich in vitamin A resulted during the years 1938–41 in a 12–15-fold increase in landings. Prices rose 40-fold, and livers were sold at from 1·5 to 13 dollars per lb. The catch then dwindled rapidly and the ‘ return per unit-effort ’ fell greatly. The ‘ bonanza ’ conditions of war-time prices and plentiful supplies brought about the most obvious over-exploitation.

I am not sure what has happened since 1946 but possibly the advent of synthetic vitamin A may have had the indirect effect of allowing the species to recover.

The search for rich sources of vitamin A and D has stimulated interest in the nutritional value of fish in many parts of the world and it is all to the good that the general biochemistry of fish is attracting more attention now. Interesting results are being obtained in all directions, the range of effort covering academic studies in comparative biochemistry to intricate technological problems. We are indebted to the organizers of the Symposium for bringing together so many different aspects of the subject.

1. SOME COMPARATIVE ASPECTS OF THE BIOCHEMISTRY OF FISH

By E. Baldwin

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A fish, according to one dictionary I have consulted, is " an aquatic, cold-blooded, water-breathing, gilled vertebrate with limbs represented by fins ". This is brief and to the point, and the definition goes on to add that " there are 12,000 species ". This is probably a gross underestimate. It will at any rate serve to remind us that, even among 12,000 species, there is plenty of room for variations of shape, size, structure, habits and even chemistry. One possible scheme of classification of the fishes is given in Table 1.

Table 1. *Classification of the fishes*

(Craniata : Gnathostomata : Anamnia)

Class PISCES

Sub-class	Order	Sub-order	Examples
CHONDRICHTHYES	Elasmobranchii	Selachoidei	Dogfishes, sharks
		Batoidei	Rays
	Holocephali		Rat-fish, rabbit fish
OSTEICHTHYES	Teleostei	Clupeiformes	Herrings, salmons
		Anguilliformes	Eels
		Ostariophysi	Carps, catfish
		Haplomi	Pikes
		Percesoses	Mullets
		Percomorphi	Perch, tunny
		Heterosomata	Flat fish
		Gadiformes	Cod, haddock
	Aetheospondyli		Ganoids
	Protospondyli		(One genus)
	Chondrostei		Sturgeons
	Polypterini		(Two genera)
	Diphoi		Lungfishes

(After Shipley & MacBride, 1920, " Zoology ", Cambridge.)

Not all of these groups will come up for discussion today, but perhaps it is well to realize from the outset that what goes for one fish does not necessarily hold for others, but rather that there are group-specific and probably even species-specific differences of a chemical as well as a morphological kind.

One striking and important phenomenon may be mentioned at once: namely that it is characteristic of the elasmobranchs that their tissues and body fluids generally contain remarkably large amounts of urea—about 2·5% in marine and about 1% in freshwater forms. This was first observed by Stadeler & Frerichs in 1858 and confirmed in the 1880's by Krukenberg. The Holocephali, which are believed on morphological grounds to be derived from an ancient and aberrant elasmobranch stock, share with the true elasmobranchs this state of physiological uraemia. So a chemical differentiation is possible between the two great sub-classes. Closely associated with this uraemia we find that, whereas arginase is confined to the liver in most fishes, amphibians and mammals, it is found in every tissue of the body in elasmobranchs and so far as the evidence goes, in the Holocephali as well. This association is probably more than fortuitous, for arginase, as every one knows, plays a central part in the ornithine cycle mechanism for urea production.

The essentially vertebrate character of the fishes is chemically demonstrated in at least two ways. First, all the fish muscles so far studied contain creatine phosphate and, if we neglect a single report of somewhat dubious origin, arginine phosphate is never present. Similarly, many fishes have been found to contain carnosine and anserine. These two aberrant dipeptides are known to occur very widely and perhaps universally in the muscles of vertebrates but, in spite of repeated attempts, have not so far been detected in invertebrates.

For some reason the distribution of carnosine and anserine has attracted a good deal of attention, and the reports in the literature serve well to emphasize my point that there are group-specific and perhaps even species-specific differences among the fishes. Clifford (1921), who studied the distribution of carnosine in the animal kingdom, stated her main conclusion in the following words: "The only relation brought out by this investigation is a morphological one. If the base is absent from one member of a zoological family, it appears to be absent from all." Thus, among the fishes, she examined four heterosomes and found carnosine absent from them all. Two gadiformes also contained no carnosine, while nineteen representatives of other sub-orders did contain it.

Quite apart from morphological considerations there is the ecological consideration of habitat. Some groups, e.g. the lungfishes, are wholly confined to fresh water; other groups again are exclusively marine, but there are a number of teleosts especially that can live in sea-water and fresh water alike. Some marine forms, such as the salmon, go into the fresh waters in order to spawn; others, such as the eel—normally a denizen of fresh water—spawn at sea. Realizing that the osmotic pressure of sea-water is at least 100 times that of fresh water, and remembering as we must that fishes *live* in water, it follows that there are large environmental differences between the two types.

I do not wish here to do more than mention a few of the ascertained facts. Fresh water has an osmotic pressure which seldom exceeds $\Delta = -0{\cdot}02°$, sea-water being about $-2{\cdot}0°$ or even more. Yet the bloods of marine fishes, apart from the Chondrichthyes, have an osmotic pressure only a little higher than that of freshwater species, about $-0{\cdot}7°$ as against $-0{\cdot}6°$.

This means little to us, who are wholly unaffected by osmotic changes in our habitual environment, but to the fishes it means a very great deal. Freshwater species live under an ever-present threat of flooding and must balance endosmosis by urinary excretion. Marine forms, on the other hand, are in constant danger of dehydration, and the exosmosis in this case is balanced by drinking. We find, looking a little further, that the glomeruli, so numerous and well developed in the kidneys of freshwater fishes, which elaborate a voluminous but very hypotonic urine, are degenerate and non-functional in marine forms, so that in these latter there is little loss of water by urine formation. But the excretion of metabolic waste products is still a primary necessity even in fishes and, in marine species which lack the usual glomerular filtration apparatus, the excretory function of the kidneys is largely taken over by the gills : some 75% of the total nitrogen excreted by marine teleosts is extra-renally excreted through this channel.

Now a large proportion—up to 30%—of the total waste nitrogen of the marine teleosts is eliminated in the form of trimethylamine oxide. Freshwater fishes, on the other hand, produce little or no trimethylamine oxide. This is why they do not smell so ‘ fishy ’ when they are dead, but the fact probably has a deeper significance. As is well known, ammonia, the predominant nitrogenous excretory product of aquatic animals in general, is a highly toxic body, requiring abundant supplies of water for its elimination if a toxic level of ammonaemia is to be avoided. Freshwater fishes have all the water that is necessary for this purpose and more ; but their marine counterparts are in a very different position. Now terrestrial or semi-terrestrial animals such as the amphibians, some chelonian reptiles and the mammals evade the dangers of ammonia poisoning by converting ammonia into urea, saurian reptiles and birds preferring to turn it into uric acid, and all these are adapted to more or less serious shortages of water. May it not be, then, that the production of trimethylamine oxide by marine, yet *never by freshwater fishes*, is in fact a chemical adaptation to the relative shortage of water to which these marine fishes are permanently subjected ? Experiments have been made which appear to suggest that trimethylamine oxide is essentially exogenous in origin, but I know of no evidence tending to suggest that freshwater fishes take a diet which is radically different from that of marine species with similar dietetic habits, or that freshwater foodstuffs are free from trimethylamine oxide and its potential precursors. Possibly this is so, but even if it is, we must still of necessity presume that there are differences in the enzymic equipment of freshwater as opposed to marine fishes. Certainly there is something peculiar here.

Another markedly peculiar fact to which I should like to make some reference is that, while vitamin A_1 forms the raw material for the prosthetic group of the retinal pigment of vertebrates generally, it is replaced in the eyes of freshwater fishes by A_2. Is this, perhaps, another aspect of freshwater *versus* marine dietetics, or is the phenomenon more deeply seated ? It is all the more striking, I think, that in euryhaline fishes, which are as much at home in the sea as they are in fresh water, A_1 and A_2 are both present, and that in those that hatch in fresh water A_2 predominates, A_1 predominating in those which are hatched at sea. Yet A_2 differs from A_1 only in containing an additional double bond in the ionone ring.

If, in conclusion, I may go back to the problem of nitrogen excretion among the fishes I would say this. The excretion of unchanged ammonia is a usual feature of aquatic animals, with exceptions only in the case of animals that have at one time lived on the land and made a subsequent, secondary return to the fresh waters, carrying their chemical adaptations with them. The replacement of ammonia by uric acid is a device that has been adopted by many terrestrial vertebrates, notably the snakes and the lizards, and by some terrestrial invertebrates also, in particular by the insects. The formation of urea from ammonia has, as I have mentioned, been adopted by those terrestrial vertebrates that did not adopt uricotelism, but seems never to have been discovered by invertebrates. In all these cases it would seem reasonable to suppose that the detoxication of ammonia, whether by conversion to uric acid or to urea, may be regarded as an adaptation to restriction of water supply. If, however, we accept this hypothesis, it remains to account for the fact that elasmobranchs excrete little or no ammonia. Probably the same is true of the Holocephali. Rather do they convert their waste nitrogen into urea, in spite of the fact that they are all aquatic. We have already seen that marine species suffer from a relative shortage of water, yet even freshwater elasmobranchs, with their superabundant supplies of water, still produce and retain urea on quite a large scale.

This is, I think, a problem worthy of the few moments I would like to devote to it, if only because, in the end, it may teach us to regard fishes as something more exalted than mere ‘ lower vertebrates ’. Marine elasmobranchs must at one time, we may suppose, have suffered from the osmotic disadvantages which still afflict their teleostean counterparts, and it would appear that they became adapted, or partly adapted, in essentially the same manner, for marine elasmobranchs, like marine teleosts, elaborate considerable quantities of trimethylamine oxide. Subsequently, it would seem, they antedated the amphibian discovery of the ornithine cycle and adopted urea as a predominant nitrogenous end product—a notable step in their chemical evolution. But subsequently they went even further: with the development of boundary membranes that are impermeable to urea, and the elaboration in the renal tubule of a special segment that reabsorbs urea from the glomerular filtrate, they became able to retain urea, to add its osmotic pressure to that exerted by the salts of their blood, and thus to raise their internal osmotic pressure to a level somewhat higher than that of the surrounding sea-water. Whether this explanation is or is not sound, the fact remains that, because so much urea is retained in the tissues, the marine elasmobranchs of the present day do indeed have an internal osmotic pressure that appreciably exceeds that of their environment, so that they are assured of a good water supply by mild endosmosis.

Finally, in those elasmobranchs which have returned secondarily to fresh water, where a high internal osmotic pressure is as great an embarrassment as a low osmotic pressure is for a marine animal, the urea content of the blood has dropped from some 2·5% to only about 1%. If this is an adaptation to freshwater existence, it is certainly a step in the right direction. But one wonders why this ‘ physiological uraemia ’ has not been altogether abandoned, for to abandon it would involve less intense endosmosis and a corresponding reduction of the work to be performed by the heart and the kidneys. But it would appear, if we may judge by the results of physiological experiments, that, after countless generations of exposure to a more or less severe degree of uraemia, the elasmobranch heart, and possibly other organs, can no longer function normally in the complete absence of urea.

These are some of the facts, and some too of the speculations, that came to mind when I was asked to address you. It may prove, as the day goes on, that they have little to do with the communications to which we are now to listen. But I would have you remember, nevertheless, that the fishes are a very heterogeneous and highly specialized group and, in a word, that, as fishes, they are as successful as we are in being men.

REFERENCE

Clifford, W. M. (1921). *Biochem. J.* **15**, 725.

2. THE PROTEINS OF FISH

By G. Hamoir

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A review of the biochemistry of fish proteins was made in 1948 by Geiger, and here more recent progress in the isolation and characterization of fish proteins will be described. No special consideration will be given to the nutritive value of these compounds or to their possible pharmacological action. It is intended in the present report to give a general picture of the composition of fish muscle and an account of the advances made in the study of the proteins of other fish tissues.

I. PROTEINS FROM SKELETAL MUSCLE

Previous work has shown that fish muscles contain myoalbumin, myogen, globulin X, myosins and stroma. The quantitative estimation of these different fractions was carried out some time ago following Smith's scheme (1937) by Reay (1935) and Reay & Kuchel (1936). At that time, however, the nature and properties of the muscle proteins were not accurately defined; a sufficiently comprehensive study of the conditions of extraction was lacking and the analysis of the protein mixtures was based only upon differences in solubility.

The influence of different factors on the extraction of fish muscle has been re-examined recently by Dyer, French & Snow (1950), and the analysis of extracts by electrophoresis has allowed a better identification of the different components (Hamoir, unpublished). As uncertainty has recently arisen concerning the nomenclature of muscle proteins, precise definitions of these based on our knowledge of rabbit muscle proteins will be given here.

Weber (1934) has given the name myogen to the albumins of the muscle juice. This definition can be generalized to the albumins of muscle extracted at low ionic strength. Recent research has shown that Weber's myogen contains the myoalbumin described by Smith (1937) (known as the component *h* of isoelectric point 4·65 by Jacob (1947, 1948)), together with a group of other proteins of much higher and fairly close isoelectric points (6·00, 6·20, 6·75) which we shall call myogens (Jacob, 1947). All these compounds exist in muscle juice or are extracted by water or dilute salt solution ($\mu \leqslant 0{\cdot}25$), together with Weber's globulin X (1934). This last fraction is removed with denatured myogens by dialysis against distilled water and is isolated by redissolution in salt solutions. Globulin X represents therefore the muscle globulins extractable at an ionic strength insufficient to bring the myosins into solution. It must not be confused with other globulins such as protein-Y of similar solubility (Dubuisson, 1950) which can only be extracted at much higher ionic strength. The general term 'myosins' includes the 'crystalline' myosin of Szent-Györgyi (1943) and the actomyosin of Banga & Szent-Györgyi (1941–1942) which is a mixture of actomyosins (according to Portzehl, Schramm & Weber, 1950 resulting from the association of actin with the 'crystalline' myosin of Szent-Györgyi. The latter is called L-myosin in the sedimentation diagrams (Portzehl *et al.*, 1950) and β-myosin in the electrophoretic patterns (Dubuisson, 1946 *a*, *b*). Stroma protein can be defined according to Smith (1937) as the residue obtained after repeated extraction by dilute hydrochloric acid or sodium hydroxide.

A. *Qualitative analysis of whole extracts*

The amounts of total nitrogen, non-protein nitrogen, myosins and non-myosins nitrogen in extracts of cod muscles have been determined by Dyer *et al.* (1950) after extraction in the cold for 3–5 minutes in a specially adapted Waring Blendor, while an analysis, using a slightly modified Tiselius electrophoresis apparatus (Dubuisson, Distèche & Debot, 1950), of extracts from carp muscles has been made by me. In my investigations the muscles were cut with a freezing microtome into slices 40 μ thick (Dubuisson, 1947) and extracted in the cold with stirring.

(a) *Extracts at ionic strength* 0·15 *and pH* 7–8. Myosins could not be detected in extracts made under the above conditions by Dyer *et al.* (1950) or by me. Of the total protein content of cod muscle 21% went into solution under these conditions and, according to Smith's analytical scheme (1937), this was made up of 6% myogen, 7% myoalbumin and 8% globulin X. Electrophoretic patterns of one-hour duration of such extracts from carp muscles showed two main components and several others present in small concentrations migrating more rapidly (Fig. 1). Fig. 1 represents a mixture of myoalbumin, myogens and globulin X. As myoalbumin has a very low isoelectric point, it can only be represented by one of the small peaks in front. It is therefore not present in these extracts in a proportion corresponding to the above analytical value of Dyer *et al.* (1950). After dialysis of the extract of Fig. 1 against distilled water, an appreciable precipitate of globulin X and denatured myogens can be removed by centrifugation. The electrophoresis of the clear solution obtained shows a general decrease of all the components except the major one, which becomes very prominent and corresponds to 80–90% of the myogens. Its mobilities in a phosphate-NaCl buffer of ionic strength 0·15 (0·1 phosphate and 0·05 NaCl) and pH 7·3 are $-3{\cdot}0\times10^{-5}$ cm.^{2}/volt/sec. on the ascending side and -2.5×10^{-5} cm.^{2}/volt/sec. on the descending side.

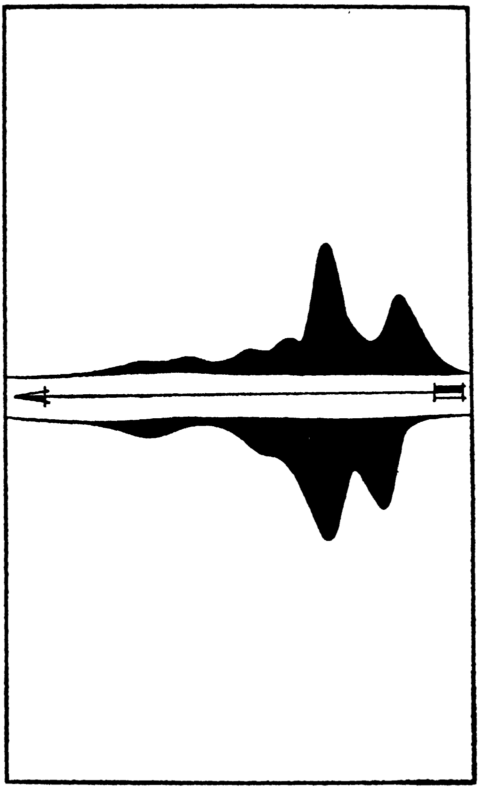

Fig. 1. Electrophoretic pattern of a one-hour extract from carp muscle at $\mu=0{\cdot}15$ and pH 7–8. Ionic strength 0·15, pH 7·3. Migration to the left. Upper part: ascending limb. Lower part: descending limb. Duration of the electrophoresis: 14,100 sec. Electrical field: 3·80 v./cm.

(b) *Extracts at ionic strength* 0·5 *and pH* 7–8. *Conditions for maximum extraction.* When the ionic strength of the extractant is higher than 0·15, structural proteins also go into solution. Extraction of the myosins is already observable at ionic strength of 0·17 in cod muscles (Dyer *et al.*, 1950); in carp muscles, no myosins are detectable in extracts made at an ionic strength of 0·35 (Hamoir, 1949), but extraction at $\mu=0{\cdot}5$ proceeds rapidly, giving turbid extracts of high viscosity and high protein content.

Fish muscle differs from mammalian muscle in its very low content of stroma proteins, these being 3% instead of 16% (Reay & Kuchel, 1936; Smith, 1937). These determinations were carried out after extraction of the pulp with hydrochloric acid (0·05 to 0·005-N) or sodium hydroxide (0·1 to 0·005-N) (Reay & Kuchel, 1936); it is desirable, however, to use a more gentle method of extraction in order to remove the myosins quantitatively without denaturation.

Some progress has been made in this direction by Dyer *et al.* (1950) by examining the influence of grinding, ionic strength, pH and nature of the extractant, temperature of extraction and cold storage.

It is most important for maximum extraction that the subdivision of the muscle fibrils must be carried as far as possible, and that any denaturation is avoided. Good recovery was obtained by these authors by homogenization of the muscles for 3–5 minutes in a Waring Blendor provided with a plate to prevent the formation of foam. The amount of residue obtained from extractions carried out in these conditions, using a solution 0·85M-NaCl and 0·02M-$NaHCO_3$ to give a pH of 7 to 7·5, varied between 3 and about 15% " depending on the conditions of the fish " (Dyer *et al.*, 1950). Varying the ratio of fish muscle to extractant solution between 1/90 and 1/18 was without influence. The nature of the salt used was of secondary importance (Table 1) and the greater differences previously observed (Smith, 1937) were probably due to an insufficient subdivision of the muscle fibrils. By the systematic study of these factors, Dyer *et al.* (1950), could separate quantitatively the myosins from the stroma at pH 7–8 by extraction with potassium iodide, magnesium chloride or sodium chloride. It is probable that similar results could be obtained by quickly freezing the fresh muscle solid with a freezing microtome, cutting it immediately in thin slices (Dubuisson, 1947) and grinding the slices with sand in the cold.

Table 1. *Efficacy expressed in per cent. of protein N extracted of normal solutions adjusted to pH* 7–7·5 *of various salts as protein extractants of cod muscle* (after Dyer *et al.*, 1950)

$BaCl_2$	$CaCl_2$	KBr	KCl	KI	K_2HPO_4	K_2SO_4	LiCl	$MgCl_2$	$MgSO_4$	NaAc	Na_3Citrate	NaCl	$NaHCO_3$	Na_2SO_4
84	87	80	82	91	86	87	86	91	83	77	83	91	77	83

Such extracts are unsuitable for electrophoretic analysis because of their high viscosity. They present all the properties of actomyosin solutions, but are from that point of view contaminated by their high content of other proteins. By altering the pH of extraction, extracts of lower viscosity can be obtained giving a general electrophoretic pattern of the different proteins present in fish muscle.

(c) *Extracts at ionic strength* 0·5 *and pH* 5–6. Extractions at high ionic strength and varying pH have been carried out by Dyer *et al.* (1950), on cod muscles. As shown in Fig. 2, the myosins begin to go into solution at pH 4·3, the extractability increasing slowly to pH 5·5 and the process is practically complete at pH 6·0. Maximum extraction is obtained between pH 7 and 9. Similar results (unpublished) were found by me with carp muscles, a shift of the curve to the right being observed with muscles kept for several weeks in frozen state. It is therefore possible, by working at pH 5–6, to get extracts containing only a small proportion of myosins. Such extracts have been analysed by electrophoresis (Hamoir, unpublished).

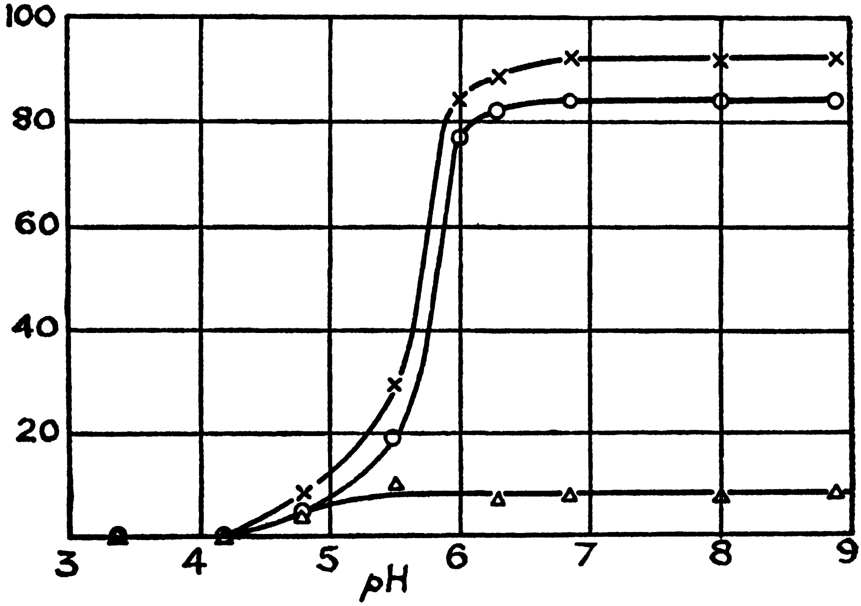

Fig. 2. Influence of the pH on the extractability of the muscle proteins of cod at ionic strength 0·85 (after Dyer *et al.*, 1950). Ordinate : per cent. of the total protein N extracted ; × —— ×, as soluble protein N ; ○ —— ○, as myosin N ; △ —— △ as non-myosin N. Abscissa : pH.

Carp muscles were extracted for ten minutes with phosphate-KCl buffers of ionic strength 0·5 (μ=0·3 KCl and 0·2 phosphate) and of pH 5·8 or 5·1. After removal of the muscles debris by centrifugation, the extracts were dialysed against phosphate-NaCl buffers of ionic strength 0·35 and pH 7·1 (μ=0·1 phosphate and 0·25 NaCl) or of ionic strength 0·15 and pH 7·3 (μ=0·1 phosphate and 0·05 NaCl).

The electrophoretic patterns obtained are given in Figs. 3 and 4. At μ=0·35 and pH 7·1, carp myosins are perfectly soluble, although they cannot be extracted at this ionic strength and pH (Fig. 3). On the ascending side, the myosins form two peaks migrating very closely together in front of the myogens, the rapid one being very sharp and the slower one showing a more symmetrical shape ; in front of these two peaks a new much more rapid component, representing only few per cent. of the protein content of the extract, can be observed. An important asymmetry exists between the ascending and the descending limbs due to the presence of the myosins : the components do not separate well on the descending side and the two peaks of the myosins are not visible. The mobilities of those different peaks have been determined ; mean values of several electrophoresis are given in Table 2.

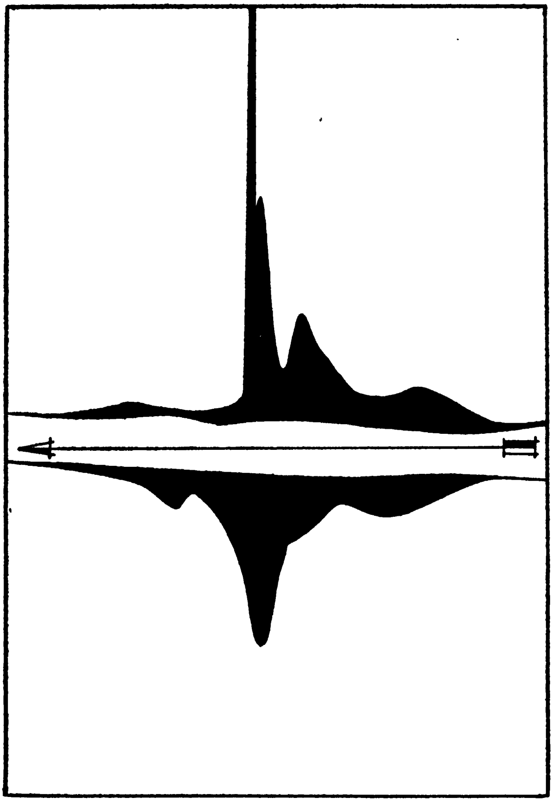

Fig. 3. Electrophoretic pattern of a ten-minute extract from carp muscle at $\mu=0{\cdot}5$ and pH 5·8. Ionic strength 0·35, pH 7·1. Migration to the left. Upper part : ascending limb. Lower part : descending limb. Duration of the electrophoresis : 79,100 sec. Electrical field : 2·0 v./cm.

By dialysis of such an extract against a solution of $\mu=0{\cdot}15$ and pH 7·3, a precipitate of myosins forms which can easily be removed by centrifugation. The composition of the supernatant is given in Fig. 4.

This electrophoretic pattern differs from the previous one obtained at low ionic strength (Fig. 1) by the presence of the peak of high mobility already observed in Fig. 3. This component behaves as a structural component : it seems not to be extractable in appreciable amount at low ionic strength, but once extracted, it is soluble at much lower salt concentrations. Its mobilities at $\mu=0{\cdot}15$ and pH 7·3 are $-6{\cdot}45\times10^{-5}$ cm.^{2}/volt/sec. on the ascending side and $-6{\cdot}0\times10^{-5}$ cm.^{2}/volt/sec. on the descending one.

Table 2. *Mobilities of the chief components of fish muscles at* $\mu=0{\cdot}35$ *and pH* 7·1 ($\mu=0{\cdot}1$ *phosphate and* 0·25 *NaCl*)

Nature of component	Mobility in 10^{-5} cm.^{2}/volt/sec.	
	Ascending value	Descending value
Quick peak	−4·1	−2·9
Myosin α	−2·88	−2·3
Myosin β	−2·78	
Myogen (major component)	−2·3	

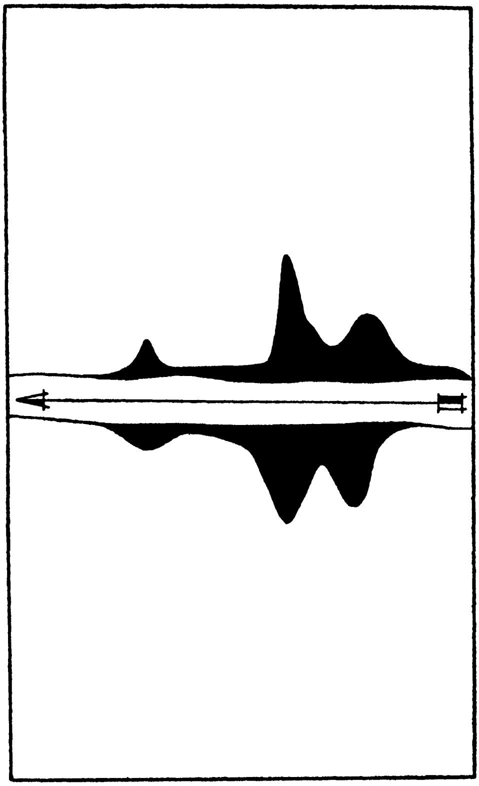

Fig. 4. Electrophoretic pattern of a ten-minute extract from carp muscle at $\mu=0{\cdot}5$ and pH 5·1. Ionic strength 0·15, pH 7·3. Migration to the left. Upper part : ascending limb. Lower part : descending limb. Duration of the electrophoresis : 16,500 sec. Electrical field : 3·70 v./cm.

The identification and characterization of the different peaks observed makes dubious the validity of the scheme of fractionation devised by Smith in 1934 and still in use. A better knowledge of the muscle components can only be achieved by their isolation.

B. *Isolation and properties of the main protein components of fish muscle*

Many of the properties of fish proteins have been described by Roth (1947).

Myoalbumin. Roth (1947) has expressed the opinion that the existence of myoalbumin is dubious. The absence in the electrophoretic patterns (Fig. 1) of a well-defined component migrating at the high mobility characteristic of myoalbumin proves that, if it exists in the extracts, its concentration must be low. It seems quite probable that the data obtained, following Smith's scheme for cod (Dyer *et al.*, 1950), are erroneous.

Myoglobin. This heme protein has been isolated in crystalline form from whale muscles by Keilin & Schmid (1948). Schmid (1949 *a*, *b*) has made a detailed study of its properties. He has found by electrophoresis that whale myoglobin like human myoglobin (Theorell & de Duve 1947) is not homogeneous, and has carried out a complete quantitative analysis of its amino-acid content (Schmid, 1949 *b*, *c*).

Myogens. Fish myogens differ markedly from mammalian myogens. The mobilities of fish and rabbit myogens are very different. Under the same conditions, the quickest main component *l* of the rabbit myogens (Jacob, 1947) migrates two to three times more slowly than the major component of the fish myogens. The latter also have a higher electrophoretic homogeneity. The difference in mobility appears not to be due to a different buffer action.

An electrotitration-curve of a fraction of haddock myogens has been made by Subba Rao (1948). The preparation used was obtained by dialysis of the muscle juice against water to remove globulin X and unstable myogens which precipitate. After centrifugation, the solution was saturated with ammonium sulphate and brought to pH 5·4. Only 50–60% of the myogens could be precipitated under these conditions. This fraction, dialysed free of salts, was used for the determinations. The curve obtained does not show any difference in buffer action when compared with rabbit myogens in the pH range 5–9 (Weber, 1927).

It is quite possible that the isoelectric points of fish and rabbit myogens are much more different than previously stated (Roth, 1947).

Globulin X. The globulin X fraction of cod muscle amounts to 8% of the total protein content (Dyer *et al.*, 1950). An analysis of haddock muscle juice (Subba Rao, 1948) shows a globulin X content of 11% of the total nitrogen of the solution and, since the remaining 89% is albumin-nitrogen, this value corresponds (see Table 4) to a globulin X content of about 2% of the total protein. Such variations are unlikely.

According to Roth (1947), the isolation of globulin X from whole fish extracts can easily be carried out by two successive dialyses, the first one against a salt solution of pH 7 and ionic strength 0·2 to remove the myosins and the second one against water to isolate globulin X. But, according to Snow (1950), the precipitation of the myosins is incomplete in such conditions, 12% remaining in solution. We have been unable to confirm Snow's results ; no myosins were observed in the electrophoretic pattern of extracts dialysed against a buffer of $\mu=0{\cdot}15$ and pH 7·3.

New determinations based upon Roth's method of isolation are necessary to know the content in globulin X of fish muscle. Since the investigations of Jacob (1947) on rabbit extracts have shown that globulin X is a very complex mixture of globulins, the values obtained depend on the method used, and an accurate description of the conditions of isolation seems particularly important.

Tropomyosin and nucleotropomyosin. Tropomyosin has been isolated from mammalian and fish muscles previously dehydrated by organic solvents (Bailey, 1948). More recently, two tropomyosins have been isolated from fish muscle by salt extraction ; one is practically identical with Bailey's tropomyosin, while the other one is an association of the same compound with nucleic acid (Hamoir, 1950). Both compounds have been found homogeneous by electrophoresis and ultracentrifugation. They crystallize in different forms. The electrophoretic mobilities are the same for both compounds and are very close to the values of the small, quick peak observed in Fig. 4. Their ultracentrifugal behaviour is very different, as shown by the sedimentation-concentration curves (Fig. 5). Their ultraviolet absorption spectra show the typical difference already observed between a protein and the corresponding nucleoprotein. The yields of the preparations of nucleotropomyosin and tropomyosin do not amount to more than 0·07 and 0·03% respectively.

Isolation of tropomyosin from muscle without previous treatment with organic solvent confirms its existence as a natural component of the fibril.

Myosin of Szent-Györgyi. Data from the literature are somewhat contradictory about the existence of this protein in fish muscle extracts. Roth (1947) could not detect its presence. The electrophoretic pattern given in Fig. 3 shows, however, the presence of two peaks corresponding to the myosins. Comparison with patterns of rabbit myosins (Dubuisson, 1946*a*) suggests that the slower peak corresponds to the β-myosin or myosin of Szent-Györgyi. Extractions of carp muscles with solutions of $\mu=0{\cdot}5$ and pH 5 allowed us to isolate by fractionation a compound having the properties of this protein. Its solution in

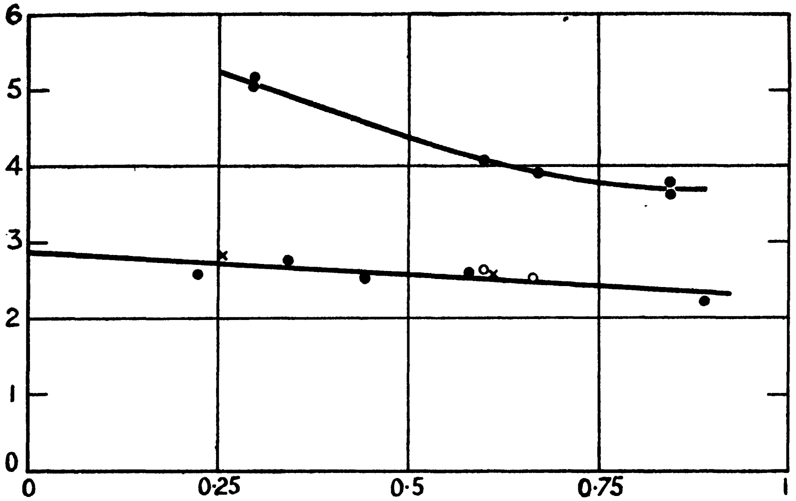

Fig. 5. Variation of S_{20} for carp tropomyosin and carp nucleotropomyosin with the concentration of the solution. Lower curve: tropomyosin. Upper curve: nucleotropomyosin.
O : values taken from Bailey *et al.* (1948) (rabbit).
X : values obtained by splitting of nucleotropomyosin at low pH.

saline was water-clear, did not show a decrease of viscosity by addition of adenosine triphosphate and was homogeneous by electrophoresis. The mobility corresponded to the value found for rabbit β-myosin (Hamoir, 1949). These results were, however, not confirmed by a further ultracentrifugal analysis. No compound sedimenting at the expected rate could be observed on a great number of extracts made in very different conditions. Addition of adenosinetriphosphate during the extraction, or shortening of the time of extraction, did not improve the results. More work is clearly necessary in this direction.

A sedimentation-concentration-curve of fish L-myosin can, however, be determined by ultracentrifugation of actomyosins in the presence of adenosinetriphosphate (Fig. 6). The solutions of twice-precipitated actomyosins were dialysed against the usual NaCl-phosphate buffer of $\mu=0{\cdot}35$ and pH 7·1 ; quantities of calcium ATP and magnesium chloride were added just before the ultracentrifugation so as to have a concentration of 0·5% calcium ATP and 0·01M-magnesium. The solutions were examined in the Svedberg oil-turbine ultracentrifuge, using the Philpot optical system (Philpot, 1938) at 60,000 r.p.m. The concentration of the proteins was determined refractometrically, before the addition of ATP, assuming a specific refractive increment of 0·00180. The values of the sedimentation constants have been corrected to 20° and to a water basis, using the partial specific volume given by Svedberg & Pedersen (1940) and the viscosity and density correction for ATP determined by myself.

Extrapolation to zero concentration gives a sedimentation constant of about 6·9, not very different from the value of 7·1 (Portzehl *et al.*, 1950) of rabbit L-myosin. Fish L-myosin seems, therefore, very similar, if not identical, with rabbit L-myosin, but the problem of its isolation from muscle extracts is still open.

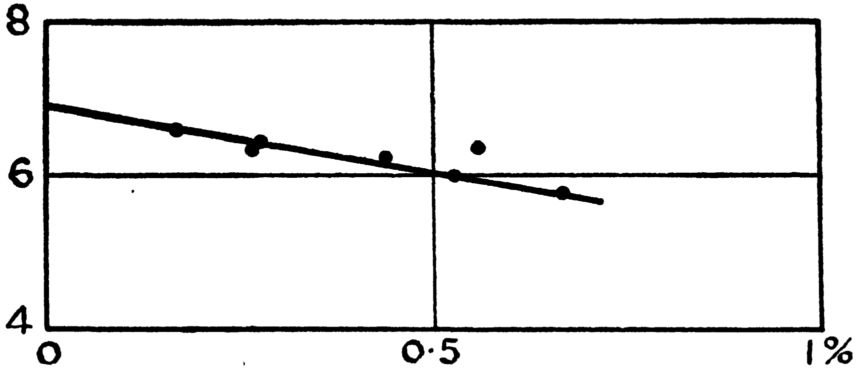

Fig. 6. Variation of S_{20} for carp L-myosin with the concentration of the solution.

Actomyosins. All the fish myosins described so far in the literature are in fact actomyosins. Some of the properties of fish actomyosins have been investigated recently. Snow (1950) has studied their denaturation by freezing. Insolubilization proceeds very quickly in the gel and slowly in the solution. Rapid freezing causes much less denaturation than slow freezing. Above the temperature of the cryohydric point of the salt used to dissolve the actomyosins, no denaturation occurs, whereas below that point denaturation is rapid. A comparison of the denaturation by freezing of actomyosin gels *in vitro* and of muscle *in situ* has been made by Subba Rao (1948). The influence of the speed of freezing and the duration of cold storage is the same in both cases, but denaturation occurs more slowly in muscle. The myogens are not influenced by freezing (Subba Rao, 1948). The value of such investigations to the preservation of fish, the elucidation of the physical state of the myosins in muscle and the curious contracture observed during thawing (Crepax & Herion, 1950) is obvious.

Subba Rao (1948) has studied the solubility and the water-retaining capacity of haddock actomyosin gels at varying pH values. A minimum is observed at pH 5·4 and a rapid increase at lower and higher pHs. Electrotitration curves of actomyosins in presence of 0·5 M-KCl from haddock, cod, lemon sole, skate and dogfish were also carried out. The maximum acid- and base-binding capacities found are given and compared with the same values for rabbit myosins (Dubuisson, 1941) in the following table (Table 3).

Table 3. *Maximum binding capacities of fish actomyosins and of rabbit myosins dissolved in* 0·5M-KCl

Species	Maximum binding capacity in 10^{-5} eq./g. protein		Authors
	Acid range	Alkaline range	
Haddock	163	165	Subba Rao (1948)
Cod	162	165	
Lemon sole	160	165	
Skate	172	177	
Dogfish	169	180	
Rabbit	156	180	Dubuisson (1941)

Some data have also been obtained by the study of the actomyosins by electrophoresis and ultracentrifugation (Hamoir, unpublished). The mobilities of twice-precipitated fish actomyosins are not influenced by the conditions of extraction (long or short duration, pH 5 or 8, fresh muscles or kept for weeks in frozen state). The mean values of many experiments are $-2{\cdot}83$ ($\alpha = -2{\cdot}88$; $\beta = -2{\cdot}78$)$\times 10^{-5}$ cm.^{2}/volt/sec. on the ascending side and $-2{\cdot}59 \times 10^{-5}$ cm.^{2}/volt/sec. on the descending one in the phosphate-NaCl buffer of pH 7·1 and $\mu = 0{\cdot}35$ previously described. If the charges of the particles are independent of the conditions of extraction, their dimensions seem to be very influenced. Extraction at $\mu = 0{\cdot}5$ and different pHs shows that the sedimentation constants of the particles decrease when the pH of extraction decreases. Several peaks can be observed in preparations made at low pH, while extractions of 10 or 60 minutes with 0·5M-KCl and 0·03M-$NaHCO_3$ (pH of extraction 7–8) always give single peak preparations. Some extractions have been made under these conditions from muscles which have been kept frozen for different periods of time ; the corresponding sedimentation-concentration-curves are given in Fig. 7.

No difference is observed between actomyosin extracted for ten minutes from fresh muscles and actomyosin extracted for sixty minutes from muscles kept frozen for different periods of time; but a definitely lower rate of sedimentation can be observed with an actomyosin extracted for ten minutes from carp muscles kept for three weeks in frozen state. If the reciprocal value of S is taken as ordinate instead of S, linear relations are obtained for each preparation corresponding to the equation $1/S = 1/S_0 + kc$. Extrapolation to $c = 0$ gives values of 1/S very near to zero or even slightly negative. Values of S_0 seem,

therefore, to be very high and cannot be determined at present. Fish actomyosins appear to be very similar to the rabbit actomyosins (Dubuisson, 1946 *a*, *b*) from the point of view of their electrochemical behaviour but notably different in their size or shape.

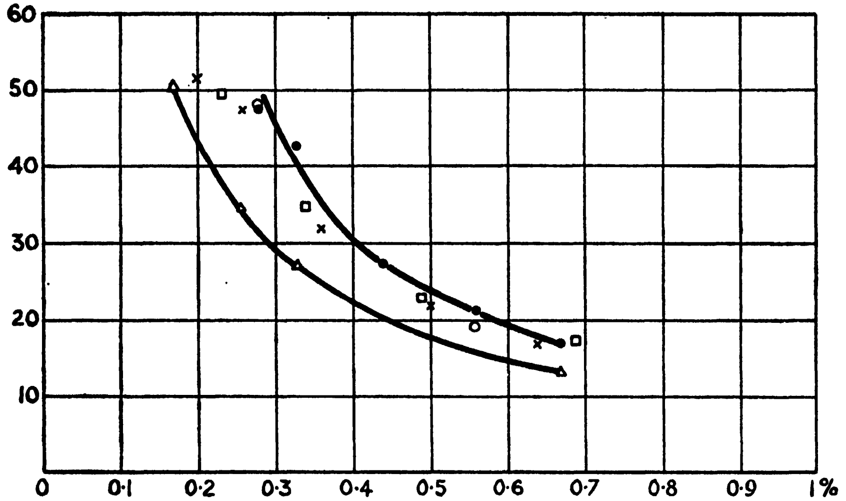

Fig. 7. Variation of S_{20} for carp actomyosins prepared by (*a*) ten-minute, or (*b*) one-hour, extraction of muscles kept frozen for different periods of time: (*a*) 2 days □; 21 days △; (*b*) 5 days × ; 24 days ○; 38 days ●.

Stroma proteins. The very low content in stroma proteins of fish muscle suggests that this residue is formed from connective tissue. As no investigation has been carried out recently on the stroma proteins, readers are referred to the section on proteins from connective tissue.

C. *Quantitative estimation of fish muscle proteins*

The two preceding sections have shown that much of the data relating to the quantitative estimation of fish proteins are dubious. Smith's fractionation scheme is out of date and some of the results obtained by this method by Dyer *et al.* (1950) or by Subba Rao (1948) are incorrect. Only the most reliable values will be given here, together with the description of the methods used. Values for mammalian muscle, obtained under similar conditions, have been added for comparison (Table 4).

All the determinations of stroma proteins have been carried out by exhaustive extractions with dilute hydrochloric acid (Smith, 1937). The albumin content of cod muscle (Dyer *et al.*, 1950) was determined by dialysis against distilled water of the extract previously diluted tenfold to remove myosins. Total protein was obtained by Subba Rao (1948) by trichloracetic precipitation of the finely minced muscle (Latapie mincer, 1 mm. holes). Different methods have been used by him for the determination of the albumins after exhaustive extraction of the muscle at pH 7 with 7% LiCl: (*a*) The extract was dialysed free of salt; the precipitate separated by centrifugation was redissolved in 10% KCl at pH 7. The residue was taken to be denatured albumin and added to the protein content of the dialysed extract. (*b*) The extract was diluted twenty times with cold water. The precipitate was redissolved in neutral 10% KCl and the residue taken to be denatured albumin, while the solution was dialysed free of salt. The protein content of the dialysed solution and the denatured albumin gave the albumin content. Good agreement was found between these two methods. The nitrogen values were expressed in protein by multiplying by 6·025.

Table 4. *Content and partition of nitrogen in fish muscle*

Species	Total N	Total coagulable N	Stroma proteins	Albumins	Authors
	in per cent. of the wet weight		in per cent. of the total coagulable N		
Haddock	2·91	2·54	3	15	Subba Rao (1948)
Cod	3·14	2·50	3	15	
Cod	2·90	2·50	3	13	Dyer *et al.* (1950)
Lemon Sole	3·03	2·75	4	16	Subba Rao (1948)
Skate	4·25	2·73	8	—	
Dogfish	4·94	2·71	10	17	
Rabbit (white muscle)	2·9	2·54	17	22	Weber & Meyer (1933)
Rabbit (white muscle)	—	—	16	9	Smith (1937)

Fish muscles differ from mammalian muscles in their very low content of connective tissue and perhaps in their content of albumins. The difference in stroma proteins presented by fishes of the elasmobranch class and the teleosts is remarkable. Much could probably be learned from other analytical determinations. As present knowledge of fish proteins is now on a safer basis for further analytical work, it is hoped that progress will soon be made in this direction. The systematic use of an improved fractionation scheme is, however, not to be recommended in view of the possibility of variation in solubility or extractability of the proteins in different species. A preliminary study of the best conditions of isolation in the case considered will always be a necessary step for accurate analytical work.

D. *Nutritive value of fish muscle proteins*

Although it is not intended to discuss this problem here, it seems worth while to point out some recent work in this direction. A book on the nutritional value of fish and the different ways in which this food can be prepared has been written by Rudolph (1946; see also Rudolf, 1947). An analysis of British Columbia fish-and-meat meals have been carried out by March & Biely (1948). The influence of canning on the amino-acid content of fish food has been investigated by Dunn *et al.* (1949), and by Neilands *et al.* (1949). This treatment apparently does not alter the amino-acids present.

II. FISH ENZYMES

The enzyme from fish tissues which has received the most attention during recent years is thiaminase, which destroys thiamine by cleaving the pyrimidylmethylene group from the thiazole portion. A short review on thiaminase has been written by Abderhalden (1947). Its distribution has been examined by Jacobson & de Avezedo (1958 *a*, *b*) and particularly by Neilands (1947). The last author has determined the thiaminase activity of muscle and viscera of a large number of aquatic animals from Nova Scotia, showing that the enzyme when present exists in both tissues. It has been found in many freshwater fishes, one marine teleost, the herring and some invertebrates. The activation and the inhibition of the enzyme by several amines and the splitting of an isoster of thiamine, called neopyrithiamine, have been described by Sealock & Davis (1949) and Sealock & White (1949). Important data could be gained in this way concerning the mechanism of action of the ferment.

A study of carbohydrate metabolism in different dolphin tissues has been made by Dubois, Geiling, McBride & Thomson (1947). The enzymic reactions examined were quantitatively the same in dolphin as in rat tissues. Adaptation of these mammals to an aquatic environment does not noticeably influence their carbohydrate metabolism. Among the enzymes of glycogenolysis, special attention has been given by different workers to the adenosinetriphosphatases. The apyrase activity of homogenized muscle tissue of different vertebrates were determined at different temperatures by Steinbach (1948, 1949). No relationship could be observed between normal environmental temperatures of the animals and the temperature coefficients found; fishes of cold and warm water showed a definitely lower coefficient than the other animals independent of their ecological milieu. The adenosinetriphosphatase activity of myosins from marine animals has been investigated by Humphrey (1949), while Roche *et al.* (1949) have isolated from carp muscles the soluble adenosinetriphosphatase recently described by Kielley & Meyerhof (1948) and have described the influence of some metallic ions on its activity and of the activity of the adenosinetriphosphatase associated with the myosins.

The optimum temperature of cholinesterase activity prepared from Torpedo's electrical tissue has been determined by Ghiretti (1948). But a much more important contribution to the cholinesterase and other esterase activity in different tissues of bony fishes, sharks and even myxine has been made by Augustinsson (1948) in an extensive study in comparative enzymology.

Since the pyloric caeca of fish possess proteolytic activity, Norman, Cooke & Chowdhury (1948) have suggested their use as substitutes for commercial bait.

III. FISH BLOOD PROTEINS

Since no general investigation appears to have been carried out recently on these proteins, only limited aspects will be considered.

The effect of temperature upon antibody production in cold-blooded vertebrates has been investigated by Bisset (1948). No lyzozyme could be found by Caselli & Melluci (1947) in fish serum. The cholinesterase and other esterase activity of the plasma and erythrocytes of different species of fish have been determined by Augustinsson (1948) in his extensive study in comparative enzymology. Papers have also been published by Fontaine and collaborators on the antigonadotropic action of the serum of the eel (Fontaine & Callamand, 1947), the iodine content of the salmon and the lamprey during migration (Fontaine & Leloup, 1950 *a*, *b*, *c* ; Leloup, 1949 *a*, *b*) and the lipase content of the salmon during migration and of different species of fish (Fontaine & Callamand, 1949 *a*, *b*).

IV. PROTEINS FROM REPRODUCTIVE ORGANS

The action of proteolytic enzymes and protaminase on salmine has been carefully reinvestigated by Portis & Altman (1947), while Block, Bolling, Gershon & Sober (1949) have described a simplified procedure for the preparation of salmine and clupein ; the purity of the preparations obtained was checked and their amino-acid content determined quantitatively. The amino-acid composition of commercial salmine has also been determined by Hamer & Woodhouse (1949).

The molecular weight of sturin prepared from the sperm of sturgeon has been determined by Plaskeev, Yaroveirko & Passynski (1948) and a value of 2,700 was found. Nuclear proteins in selachians have been investigated by Cardoso & Piro (1947).

The pharmacological action of these proteins will not be considered here.

V. PROTEINS FROM CONNECTIVE TISSUE

Very little is known about the proteins which form the connective tissue. However, some progress has been made on fish collagen, for Salo (1948) has determined its arginine and histidine content, while Loofbourow, Gould & Sizer (1949) have measured its absorption spectrum. No absorption characteristic of phenylalanine, tyrosine and tryptophan could be detected. Collagen from the skin of the whale has been isolated by Ichimaro (1944) and some analyses have been carried out on this protein. A method of preparation and crystallization of procollagen from different sources mainly from the skins of different animals has been given by Orekhovich & Plotnikova (1948) and Orekhovich, Tustanovsky & Plotnikova (1948). The highest yields of procollagen are obtained from fish skins, but its isolation from the swim-bladder has also been reported. The isolation of gelatin and a collagen-like protein from herring scales has been described by Block, Horwith & Bolling (1949).

COMPARATIVE BIOCHEMISTRY OF FISH PROTEINS

Many of the comparative studies which have been made on fish proteins concern mainly the enzymes, and as they involve very special problems and are more closely related to comparative metabolism than to the comparative biochemistry of fish proteins, they will not be considered here. Very little seems to have been done so far on the comparative study of tissue proteins. Apart of the work of Labhart, Süllman & Viollier (1947) on lens proteins, comparative studies in this field have only been carried out on muscle (Jacob, 1945 ; Cigada, Citherio, Ranzi & Tosi, 1948 ; Subba Rao, 1948 ; Hamoir, 1949). A first contribution to the comparative biochemistry of tissue proteins can now be attempted by comparison with the muscle proteins of different classes of vertebrates.

The scientific importance of the study of fish proteins becomes still more evident when considered from this angle. As fish represent the lowest classes of vertebrates and mammals the highest, a comparison of fish and mammalian proteins should allow one to draw very general conclusions on the proteins of the vertebrates. Such a comparison will also contribute to bridge the gap existing between our knowledge of muscle chemismus and physiology based upon studies on frogs and of muscle protein chemistry which has developed mainly from studies on rabbits.

In comparing different animals which have been more or less completely studied (fish, amphibia, mammalia), it is important to make the distinction between qualitative and quantitative variation. One qualitative difference only has so far been observed and this concerns the myogens ; this has already been described in the case of carp and rabbit muscle. Data concerning frog myogens (Jacob, 1945; Roth, 1947) are still very imcomplete, but they show that this group of proteins is much more related to the fish myogens than to the mammalian myogens. An important distinction can therefore be made between cold-blooded and warm-blooded vertebrates.

Other differences of minor importance are observed between fish and frogs or cold-blooded and warm-blooded vertebrates. The formation of actomyosins during the extraction is much quicker in the case of fish muscle than in the case of any other animal (Guba, 1943). The proportion of stroma proteins in muscle, the content of the myosin of Szent-Györgyi in the extracts, and the differences in sedimentation constants of the actomyosins allow us also to characterize the muscle proteins. But striking similarities are also observed. Fish myosins and the rabbit myosins have the same electrophoretic mobilities. Fish tropomyosin has been found to be practically identical with rabbit tropomyosin by the different methods used (Bailey, 1948; Hamoir, 1950). The first lineaments of a common protein pattern underlying muscular contraction is beginning to appear through the wealth of data already acquired.

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3. THE CHEMISTRY AND METABOLISM OF THE NITROGENOUS EXTRACTIVES IN FISH

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INTRODUCTION

The study of the nitrogenous extractives in various representatives of the animal kingdom, including those from aquatic environments, has proved to be one of the most interesting chapters in the history of comparative biochemistry. Such investigations were originally undertaken in order to gain deeper insight into intermediate protein metabolism and also possibly to extend the knowledge of animal poisons, but the results obtained have been of much wider significance than at first imagined.

As a result of the work of the Hoppe-Seyler-Ackermann school in Germany, and equally active ones in Russia and Japan, a considerable body of knowledge has been built up (Guggenheim, 1940) which has made possible the study of comparative biochemistry, and readers of Baldwin's (1937, 1947) books must agree that the subject is one of ever-widening interest.

In such studies, marine animals, including fish, have occupied an important place.

In presenting this short review of the nitrogenous extractives, and in particular the nitrogenous bases in fish, two points must be made clear at the outset: (1) the approach owing to the author's personal interests has had to be almost exclusively a chemical one; it would have required a physiologist intimate with the large, scattered and often contradictory data to present a balanced picture of the rôle many of these compounds play in the metabolism of fish, so that little will be attempted in this direction; (2) the data presented are concerned almost exclusively with the skeletal muscle, chiefly because more exist on this aspect than on any of the other body components.

THE AMOUNT AND NATURE OF THE EXTRACTIVES

The nitrogenous extractives, on the whole, form a relatively small proportion of the total nitrogen in fish muscle. In some species, e.g. flat fish and gadoids (cod, haddock, etc.) they form about 9–14% of the total nitrogen (Table 1), and in the herring group, 14–18%; whilst in the elasmobranchs (skate, dogfish, etc.) they amount to 34–38%. The figures given probably vary considerably even within the same species, due to a variety of causes, such as age, sex, season or spawning-cycle. The latter, for example, is said to cause an increase in the non-protein nitrogen in salmon muscle (Green, 1919). These bases, although relatively small in amount, and contributing little to the food value of fish, are important in many other ways. Some almost certainly contribute to the flavour of fish and are said to be of dietetic importance in relation to the secretion of digestive juices (Komarov, 1933; Campbell, 1935). The ease with which many are attacked by micro-organisms affects in turn the relative keeping quality of the various species (Shewan, 1938, 1949), and, as will be shown later, the nature and amounts of their decomposition products may in fact determine whether a fish is edible, or even whether it is poisonous or not. Indeed, Kimata (1941) has shown that the speed of decomposition of various species is dependent almost wholly on the nature of the extractives, the muscle proteins all being attacked at approximately the same rate. Thus, bonito and mackerel spoil much faster than sea bream and halibut, lobster spoiling the fastest of all.

The extractives present in fish and crustacean muscle may be grouped as follows :—

The volatile bases, including ammonia, mono-, di- and trimethylamines.

Trimethylammonium bases, e.g. quarternary alkylamines (trimethylamine oxide) ; betaines.

Guanidine derivatives, such as creatine and arginine.

Imidazole or glyoxaline derivatives, such as histidine, carnosine and anserine.

A miscellaneous group, including urea, amino-acids and purine derivatives.

With regard to the latter group, although it is known that certain amino-acids, notably glycine and alanine (Kutscher & Ackermann, 1936) and purines (Kutscher & Ackermann, 1933 *b* ; McCay, 1937), are invariably present in the extractives, data are so few that they are excluded from the present discussion. Moreover, the volatile base fraction is usually small in very fresh fish (Shewan, 1937, 1938, 1949), now under discussion, although it becomes of increasing importance as the fish spoils. Further reference to these will be left to the end.

I. TRIMETHYLAMMONIUM BASES

(a) *Trimethylamine oxide.* From many points of view this is one of the most important bases present in fish muscle and one for which most data are available. Unlike urea, trimethylamine oxide was known as a synthetic chemical entity (Dunstan & Goulding, 1896, 1899 ; Hantzsch & Hilland, 1898) some 13 years before being isolated by Suwa (1909) as a natural product from dogfish muscle. Earlier investigations appeared to indicate that this compound was confined wholly to marine species (Kutscher & Ackermann, 1933 *b*), it being

Table 1. *Total nitrogen, protein nitrogen and non-protein nitrogen in fish*

Species	No. of samples	Total nitrogen %	Protein nitrogen %	Non-protein nitrogen %	Non-protein nitrogen as % of total nitrogen	Author
ELASMOBRANCHS						
Dogfish (*Acanthias vulgaris*)	3	3·29–3·96	1·95–2·49	1·05–1·34	34·0	Shewan
		(3·48)	(2·16)	(1·18)		
Blue Skate (*Raja batis*)	1	3·62	2·17	1·20	33·0	
Cuckoo Ray (*Raja circularis*)	1	3·98	2·57	1·34	33·7	
Thornback Skate (*Raja clavata*)	1	3·58	2·18	1·38	38·6	
Rays (sp. ?)	2	3·80–4·03		1·38–1·42		Boury & Schvinte (1935)
		(3·92)	(2·52)*	(1·40)	35·6	
TELEOSTS						
Gadoids						
Cod (*Gadus morrhua*)	1	2·86	2·43	0·41	14·3	Shewan
	1	2·83	2·47	0·36*	12·7	Reay *et al.* (1946)
Haddock (*Gadus aeglefinus*)	15	2·56–3·02	2·14–2·83			
		(2·80)	(2·48)	(0·37)	13·0	
Whiting (*Gadus merlangus*)	1	3·0	2·66	0·34	11·3	Shewan
	2	2·81–2·86	2·45–2·48	0·34–0·38		Boury & Schvinte (1935)
		(2·84)	(2·47)*	(0·36)	12·7	
Ling (*Molva molva*)	1	3·03	2·74	0·34	11·2	Shewan
Flatfishes						
Plaice (*Pleuronectes platessa*)	2	2·70–2·78	2·23–2·34	0·30–0·31		Shewan
		(2·73)	(2·88)	(0·31)	11·3	
Lemon Sole (*Pleuronectes microcephalus*)	3	2·76–3·14	2·27–2·59	0·30–0·34		
		(2·93)	(2·47)	(0·33)	11·3	
Dab (*Pleuronectes limanda*)	1	2·85	2·38	0·38	13·3	
	2	2·88–2·93		0·24–0·29		Boury & Schvinte (1935)
		(2·90)	(2·74)*	(0·265)	9·2	Boury (1936)
Megrim (*Arnoglossus megastoma*)	1	3·07	2·96	0·30	9·7	Shewan
Clupeids						
Herring (*Clupea harengus*)	4	2·70–3·01	2·36–2·63	0·34–0·40		Boury & Schvinte (1935)
		(2·90)	(2·53)*	(0·38)	13·1	
	Combined sample 6 fish	2·98	2·67	0·41	13·7	Shewan
Sardine (*Clupea pilchardus*)	3	3·27–3·78	2·83–3·19	0·44–0·59		Boury (1936)
		(3·46)	(2·97)*	(0·49)	14·1	
Herring (Finnish Gulf—*Clupea harengus membras*) Female	?	2·44–2·73		0·34–0·51		Levieva (1950)
		(2·60)	(2·15)*	(0·45)	17·3	
Male	?	2·42–2·74		0·31–0·60		
		(2·57)	(2·10)*	(0·47)	18·3	
Miscellaneous species						
Mackerel (*Scomber scombrus*)	Combined sample 3 fish	3·33	2·76	0·42	12·5	Shewan
	7	2·90–3·10	2·39–2·76	0·38–0·51		Boury & Schvinte (1935)
		(3·05)	(2·60)*	(0·45)	14·8	Boury (1936)
Grey Gurnard (*Trigla gurnardus*)	1	3·20	2·87	0·30	9·4	Shewan
Horse Mackerel (*Caranx trachurus*)	1	3·12	2·68	0·35	11·2	
Monk fish (*Lophius piscatorius*)	1	2·70	2·40	0·29	10·7	
Hake (*Merluccius merluccius*)	2	2·76–2·90	2·49–2·58	0·32–0·34		
		(2·84)	(2·54)	(0·33)	11·6	
Catfish (*Anarrhicas lupus*)	1	2·56	2·29	0·26	10·0	
Golden Perch (*Sparus auratus*)	1	3·22	2·85*	0·37	11·5	Boury (1936)

* By difference.

[*To face p.* 28.

said to play a rather specific rôle in osmo-regulation of certain species (Hoppe-Seyler, 1929); but more recent results appear to show that it can occur in both freshwater fishes (Cook, 1931, and private communication; Reay, 1938; Lintzel, Pfeiffer & Zippel, 1939) and amphibia, (Wilson & Wolff, 1938), albeit in much smaller quantities than in marine types, so that its physiological rôle, as will be mentioned later, is probably of much wider significance than at first thought.

Table 2. *The distribution of trimethylamine oxide in the marine world* [Mainly after Norris & Benoit (1945); Dyer (private communication) and Shewan (unpublished results).]

	Trimethylamine oxide in mg. %
I. PLANTS	
Diatoms and algae*	0
II. ANIMALS	
Echinoderms:	
(1) Pacific sea cucumber	0
(2) Atlantic sea cucumber	480
(3) Atlantic starfish	0
Molluscs:	
(1) Pacific and Atlantic clams, mussels and oysters	0
(2) Pacific scallops	225–580
(3) Atlantic scallops	480
(4) Pacific amphineura and gastropods	0
(5) Pacific squid	800–830
(6) Atlantic squid	365–390
Arthropods (crustacea):	
(1) Pacific copepods	120–450
(2) Decapods:	
(*a*) Pacific crabs	70–350
(*b*) Pacific shrimps	210–470
(*c*) Atlantic lobsters	150–650
Fishes:	
(1) Elasmobranchs:	
(*a*) Pacific dogfish	660–1010
(*b*) Atlantic dogfish	710–1430
(*c*) Atlantic skates and rays	250–1430
(2) Teleosts:	
(*a*) Gadoids:	
Atlantic cod, haddock and whiting	100–1080
(*b*) Flat fishes:	
Atlantic plaice, sole, dabs, halibut, etc.	110–600
Pacific flounders	140–160
(*c*) Clupeids:	
Atlantic herring	215–315
Mammals:	
Porpoise	0·5
Whales	0–120

* Trimethylamine is present in seaweeds and algae, but absent in freshwater algae. (Kapaller-Adler & Vering, 1932.)

Even in marine organisms it shows a somewhat erratic appearance, being absent in diatoms and algae (Reay, 1938 ; Dyer, 1950, private communication), certain echinoderms (compare Pacific and Atlantic sea cucumber) and molluscs (Table 2), but is present in varying amounts in all crustacea and fishes so far examined. With regard to the latter, it will be noted that the trimethylamine oxide content is highest in the elasmobranchs, lowest in the flat fishes, the gadoids and clupeids being intermediate. The much lower values in freshwater species compared with marine types is obvious from Table 3, although exceptions do occur, as Cook (1931) found in salmon, the land-locked variety having more than three times that of the same species from the sea.

Table 3. *Comparison between fresh- and salt-water species in respect of trimethylamine oxide content*

[Mainly after Cook (1931) ; Reay (1938) ; Lintzel *et al.* (1939).]

FRESH WATER		SALT WATER	
Species	Trimethyl-amine oxide in mg. %	Species	Trimethyl-amine oxide in mg. %
FISH :		FISH :	
Ling (Lake Ontario)	116	Cod	470
		Haddock	390
		Pollock	190
Alewife (river herring—Lake Ontario)	185	Herring	250
Salmon (land-locked)	83	Salmon	25
King salmon (fresh-water fingerling)	0–4·5	King salmon (adult in salt water)	50–60
Lake trout	66	Smelt	340
River trout	9–73		
Whitefish (*Coregonus* sp.)	0		
Cisco (Lake Ontario) (*Leucichthys* sp.)	11		
Bream, perch, pike, etc.	5·0–92·0		
CRUSTACEA :		CRUSTACEA :	
River crayfish	13–50	Shrimps, crabs, lobsters	70–650
Zooplankton (mainly daphnia and coelsphaerium)	0	Zooplankton (mainly decapod larvae)	250

AMPHIBIA

Necturus (trimethylamine oxide present) (Wilson & Wolff, 1938)

Mammals, such as porpoises and whales, also have a small amount of trimethylamine oxide in the muscle (Dyer (1950), private communication ; Robinson & Ingram (1950), private communication).

Several factors appear to affect the average content of trimethylamine oxide in marine species. One of these is undoubtedly environment, as has already been pointed out in comparing the Pacific and Atlantic

Table 4. *Comparison between North Sea and Arctic water fish in relation to trimethylamine oxide content*

Species	NORTH SEA			ARCTIC		
	No. of samples	Trimethylamine oxide content in mg. %		No. of samples	Trimethylamine oxide content in mg. %	
		Limiting values	Average		Limiting values	Average
I. ELASMOBRANCHS						
(*a*) Skate (various sp.)	5	250–640	480	5	417–680	540
(*b*) Dogfish	4	710–1035	925	2	805–1020	930
II. GADOIDS						
(*a*) Cod	33	220–390	330	260	100–1080	550
(*b*) Haddock	135	102–275	235	36	320–750	430
(*c*) Coalfish	1	213	213	13	300–525	415
III. FLAT FISH						
(*a*) Plaice	2	110–130	120	3	190–360	280
(*b*) Dab	3	160–200	180	4	370–570	440
IV. MISCELLANEOUS						
(*a*) Catfish	2	250–260	255	8	210–420	330

sea cucumber. This effect is perhaps more clearly shown in comparing various species of fish from the North Sea and the Arctic waters (Table 4) (Shewan (1950), unpublished results). Clearly in several instances, too few samples have been examined, but, excluding the elasmobranchs, the general trend is for the Arctic fish to have much higher values than North Sea fish (cf. cod and haddock). A seasonal variation has also been noted by Norwegian workers (Ronold & Jakobsen, 1947) for herring (Fig. 1) and sprats (Fig. 2), and by Lintzel *et al.* (1939) for some freshwater species, although no such variable effect has been detected for North Sea cod, haddock and whiting caught off Aberdeen (Shewan (1949), unpublished results). The size or age of the fish (cod, haddock and whiting) also appears to affect the average trimethylamine oxide content, the larger fish having the greater concentration (Table 5), but wide variations are to be found even within the one size or age group.

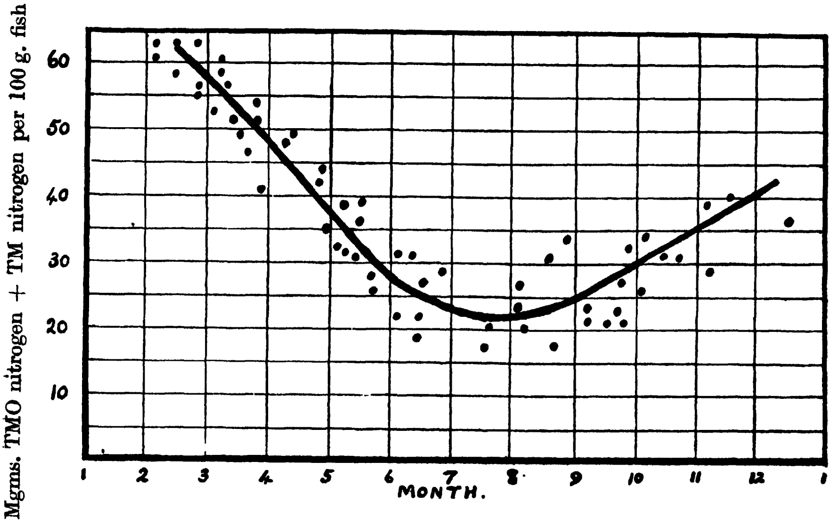


Fig. 1. Seasonal variation in trimethylamine oxide (TMO) content (trimethylamine (TM) + trimethylamine oxide) of herring (*Clupea harengus*). (After Ronold & Jakobsen.)

A similar relationship between age or size and trimethylamine oxide content has been found by Dyer (1950, private communication) in the Canadian yellowtail (*Limanda* sp.) but not in cod. Even different muscles may have different trimethylamine oxide contents, the dark lateral line of the flesh of both herring and tunny having only about half of that of the rest of the skeletal muscle (Shewan, unpublished results, 1950). Godsil (1941) has already recorded the fact that in spoiling tuna, trimethylamine accumulates in certain parts of the skeletal muscle and not in others, and although no explanation was offered, the differences might well be due to initial differences in the content of trimethylamine oxide.

Amongst the more interesting properties of trimethylamine oxide, the following may be mentioned. It acts as a weak base, forming salts with acids, e.g. HCl; these salts have a marked buffering action in the region of pH 4·5; the base can act as a hydrogen acceptor being reduced, e.g. by certain bacteria (*Micrococci*, *Achromobacter*, *Flavobacter*, *Pseudomonas*, etc.) (Beatty & Gibbons, 1937; Tarr, 1939, 1940; Watson, 1939*a*,*b*), as in spoiling fish, or by glutathione (Ackermann, Poller & Linneweh, 1925) in the muscle system, to trimethylamine. Moreover, it is reduced either in acid or in alkaline

Table 5. *The effect of length on the average value of trimethylamine oxide in cod, haddock, and whiting muscle*

Length	No. of samples	Trimethylamine oxide in mg. %
COD:		
Up to 50 cm.	31	500
50–60 cm.	69	518
60–70 cm.	102	560
70–80 cm.	40	605
80–100 cm.	20	613
HADDOCK:		
25–30 cm.	85	207
30–35 cm.	32	263
WHITING:		
20–25 cm.	22	235
25–30 cm.	39	290
30–35 cm.	9	303

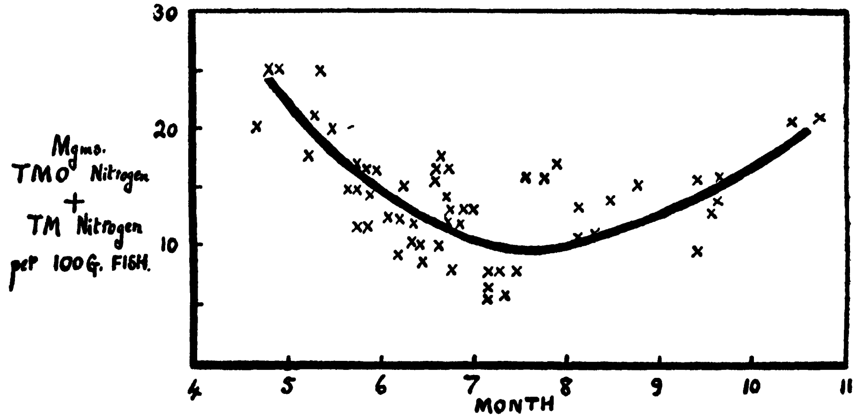


Fig. 2. Seasonal variation in trimethylamine oxide (TMO) content (trimethylamine + trimethylamine oxide) of brisling (*Clupea sprattus*). (After Ronold & Jakobsen.)

solutions by nascent hydrogen to trimethylamine by Devarda's alloy, Zn, Al (Lintzel, 1934) and $SnCl_2$ (Cook, 1931); this reduction is also quickly effected at room temperature in 50% ethanol-water by $TiCl_3$ (Ronold & Jakobsen, 1947). All these reduction methods have been used as a basis for estimation.

In the presence of certain catalysts or by heating to a high temperature (Dunstan & Goulding, 1899), trimethylamine oxide is broken down to dimethylamine and formaldehyde, as a result of which canned (Lunde & Mathiesen, 1934) and other marine products (Reay, 1936) have frequently much higher contents of formaldehyde than the fresh fish. In this connection, it may be of interest to mention that owing to the presence of trimethylamine oxide, considerable amounts of bisulphite can escape detection by the usual techniques, presumably owing to the formation of the bisulphite—formaldehyde complex (Shewan, 1948).

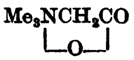
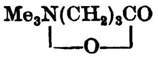
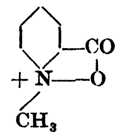
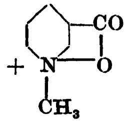
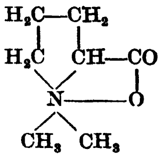
Recent Norwegian experience has also shown that trimethylamine oxide is the main constituent causing the corrosion of tinned cans of certain marine products (Jakobsen & Mathiesen, 1946; Ronold & Jakobsen, 1947).

Along with betaine and other trimethylamine compounds, trimethylamine oxide is said to be responsible for the production of 'fishy' flavours in various foodstuffs, e.g. milk and butter, liver oils, cured fish, cold-stored and CO_2 stored fish. In every instance in which 'fishiness' occurs, a fat is present along with trimethylamine or its oxide or compounds which can give rise to them (choline, lecithin, etc.) and it has been suggested (Davies & Gill, 1936) that the trimethylamine, owing to its ease of oxidation, takes part in reactions involving the transfer of nitrogen into organic combination with the fat breakdown products to form fat soluble 'fishy' flavoured products.

The origin of trimethylamine oxide is still in doubt. Some believe it to be the non-toxic end-product of protein metabolism (Hoppe-Seyler, 1929, 1930; Stransky, 1933), although so far there has been no clear demonstration that fish tissues, particularly of the marine varieties, have enzymes capable of oxidizing trimethylamine to trimethylamine oxide (Kapeller-Adler & Vering, 1932). In man and other mammals, on the other hand, such systems do exist (Lintzel, 1934; Tarr, 1941; Norris & Benoit, 1945). In some species, e.g. salmon, in which the trimethylamine oxide increases considerably in a marine environment, food containing the oxide is said to be the ultimate source. Thus, Benoit & Norris (1945) found young salmon, containing little or no trimethylamine oxide, on transference to salt water and being fed on a trimethylamine oxide-free diet showed no increase in the oxide content of the muscle. On feeding a diet containing the oxide, some retention, however, was found.

The physiological rôle of trimethylamine oxide is also in doubt. If, as just mentioned, it is of exogenous origin, it may be of no importance physiologically. In Smith's opinion (1936), the apparent retention, by marine teleosts at any rate, may simply be due to the fact that it is excreted exclusively in the urine (some 30% of the urinary nitrogen is accounted for in some teleosts as trimethylamine

Table 6. *The occurrence of betaines in marine animals*

	Species	Betaine content in mg. %	Author
I. Glycine betaine  Me_3NCH_2CO	Dogfish (adults)	70	Berlin & Kutscher (1924)
	Dogfish (embryos)	120	
	Rays	210	Kapeller-Adler & Krael (1930)
	Sharks	260	
	Cod	102	
	Witch	75	Hoppe-Seyler & Schmidt (1927)
	Carp	10	Kapeller-Adler & Krael (1930)
	Pike	14	Lintzel *et al.* (1939)
	River lamprey	54	Strack *et al.* (1937)
	Edible mussel	+*	Ackermann (1921)
II. γ-Butyrobetaine  $Me_3N(CH_2)_3CO$	Eel	10	Hoppe-Seyler & Schmidt (1927)
	Noah's ark shell	+	Kutscher & Ackermann (1933 *b*)
III. Homarine 	Lobster	+	Hoppe-Seyler (1933)
	Mussel (California) liver	+	Riegel *et al.* (1949)
	Noah's ark shell	+	Kutscher & Ackermann (1933 *a*)
IV. Trigonelline 	Sea urchin	+	Holtz *et al.* (1924)
	Jelly fish	+	Haurowitz & Waelsch (1926)
V. Stachydrine 	Noah's ark shell	+	Kutscher & Ackermann (1933 *b*)

* Known to be present—quantities unknown.

oxide (Grollman, 1929)), at least it does not diffuse through the gills as does urea, and that the rate of urine formation in these fishes is extremely small.

In the elasmobranchs, on the other hand, trimethylamine oxide almost certainly plays an important rôle in osmoregulation (Hoppe-Seyler, 1929, 1930; Smith, 1936; Krough, 1939) in all body tissues and fluids where its concentration is high. The most recently expressed view and one already hinted at by Hoppe-Seyler (1929) is that the rôle of trimethylamine oxide in fish ought to be sought in that of a methyl donor (Barrenscheen & Pantlitschko, 1949), the evidence being that trimethylamine oxide plays the same part in transmethylation of choline as homocysteine does for methionine (see also Bach 1945).

(b) *Betaines.* The present, rather scanty data on the distribution of the betaines is given in Table 6. It will be noted that glycine betaine parallels trimethylamine oxide, for in marine species the elasmobranchs contain the greatest, and the flat fish the least amounts, whilst freshwater species have much less than the marine types.

It may be of interest to mention here that in marine invertebrates (molluscs, crustaceans) several interesting betaines have been isolated, such as homarine, trigonelline, stachydrine, etc. So far, none of these have been shown to be present in fish muscle and little is known about their metabolism, although they may possibly play a part in biological methylation.

II. GUANIDINE DERIVATIVES

Creatine and Arginine. The available data for these rather important muscle constituents, whose physiological rôle is now well established, are given in Table 7. In general, it will be seen that the creatine content of the skeletal muscle of fish varies greatly, not only in different species but also between individuals of the same species (see also Tarr, 1950). In contrast with trimethylamine oxide, there appears to be with the betaines and, as will be shown presently, urea, no systematic difference between elasmobranchs and teleosts or marine and freshwater species.

In crustacea and shell fish, only traces of creatine have been found, because arginine replaces creatine in the phosphagen of these species (Kutscher & Ackermann, 1933*b*).

III. IMIDAZOLE OR GLYOXALINE DERIVATIVES

(a) *Anserine and Carnosine.* In a previous review on the distribution of these compounds in marine and freshwater animals (Reay, Cutting & Shewan, 1943) it was pointed out that few reliable data existed, particularly in view of the criticism of some of the methods used for differentiating carnosine and anserine. It was believed to

Table 7. *Distribution of creatine in the muscle of fish and crustacea expressed as per cent of the wet muscle*

Common name	Systematic name	European species (creatine %)	Canadian and American species (creatine %)	Author
ELASMOBRANCHS :				
Shark	*Sphyrna zygaena*	0·401	—	Kisch (1930)
Dogfish	*Squalus sucklii*	—	0·600	Hunter (1929)
	Acanthias vulgaris	0·460	—	Eggleton & Eggleton (1928)
Skate	*Raja clavata*	0·280	—	Mellanby (1907)
		0·339*	—	Bate-Smith (1942)
		0·440	—	Eggleton & Eggleton (1928)
	Raja stabuliformis	—	0·337	Campbell (1935)
	Raja binoculata	—	0·481	Hunter (1929)
Ray	*Torpedo ocellata*	0·287	—	Kisch (1930)
TELEOSTS :				
Cod	*Gadus morrhua*	0·350	—	Mellanby (1907)
		—	0·576	Campbell (1935)
Haddock	*Gadus aeglefinus*	0·478*	—	Bate-Smith (1942)
	Melanogrammus aeglefinus	—	0·639	Campbell (1935)
Herring	*Clupea harengus*	—	0·577	
	Clupea palasii	—	0·740	Hunter (1929)
Salmon	*Salmo salar*	—	0·602	Campbell (1935)
	Oncorhynchus tschawytscha	—	0·618	Okuda (1912)
Sea wrasse	*Labrus turdus*	0·423	—	Kisch (1930)
CRUSTACEA :				
Lobster	*Homarus americanus*	—	0·005	Campbell (1935)
	Homarus vulgaris	0	—	Eggleton & Eggleton (1928)
Crab	—	arginine instead	—	Meyerhof & Lohmann (1928)
FRESHWATER FISH AND CRUSTACEA :				
Burbot	*Lota vulgaris*	0·526	—	Palladin & Wallenberger (1915)
Carp	*Cyprinus carpio*	—	0·487	Okuda (1912)
		0·350–0·460	—	Palladin & Sigalova (1935)
Crayfish	(*Astacus fluviatilis*) (?)	arginine instead		Kutscher (1914)
SHELLFISH :				
Mussell	*Mytilis* (adductor muscle)	0	—	Eggleton & Eggleton (1928)
Mollusc	*Pecten*	0	—	

* Muscle juice.

have been established, particularly from the work of Ackermann & Hoppe-Seyler (1931), that anserine was present in shark, cod and conger eel, and carnosine only in the freshwater eel, neither being present in herring and perch. Within the last year, a most important contribution has been made to this subject by Yudaev (1950) who, using a partition chromatographic technique, claimed to be able to separate and specifically identify histidine, carnosine and anserine. (This technique, by the way, is a good example of the successful application of the newer methods to old problems; see also Brante (1948) for the separation of betaine, trimethylamine oxide and other bases; and Riegel, Stranger, Wikholm, Mold & Sommer (1949) for the separation of the bases in shellfish). By this method, Yudaev has shown (Table 8) that in freshwater fish neither carnosine nor anserine is present, although histidine is there in fair quantity; carnosine is present in some species (e.g. sturgeon), whilst anserine is present in the wholly marine species (cod). Applying this technique, which appears to work satisfactorily, to our North Sea fish, it would appear that in cod, haddock and whiting, anserine is present and possibly carnosine in cod only. In skate and herring anserine and possibly carnosine are both absent.

Table 8. *Content of histidine, carnosine and anserine in fish muscle (mg. per* 100 *g. wet tissue*) (*after Yudaev,* 1950)

Species	Histidine	Carnosine	Anserine
Carp (*Cyprinus carpio*)	241·3	0*	0*
Chub (*Squalius cephalus*)	217·6	0	0
Dace (*Leuciscus leuciscus*)	142·4	0	0
Gudgeon (*Gobio gobio*)	229·6	0	0
Loach (*Gobitis taenia*)	127·2	0	0
Perch (*Perca fluviatilis*)	217·6	0	0
Pike-perch (*Lucioperca lucioperca*)	90·0	0	0
Sturgeon (*Huso huso*)	0	306	0
Sturgeon (*Acipenser ruthenis*)	0	211·9	0
Cod (*Gadus callarias*)	0	0	150
CARP:			
Muscle (average 5 experiments)	242		
Blood (do.)	3		

* 0 signifies <3 mg. %.

(b) *Histidine.* As mentioned above, histidine can exist in quantities varying from 127–240 mg. % in the muscle of various species of freshwater fish, although little or none is present in the blood (Table 8). It is now recognized that histidine is also a normal component of the muscle extractives of several marine species—dogfish, mackerel, sardine and tunny (Spahr, 1937; Igarasi, 1938, 1939; Johnson & Johnson, 1939; Geiger, Courtney & Schnakenberg, 1944). In the last fish, 470 mg. % have been recorded (Suzuki & Yoshimura, 1909) and indirect evidence shows that it is present in amounts up to 200 mg. % in mackerel (Geiger, 1944; Geiger *et al.*, 1944). During spoilage, histidine is decarboxylated by bacteria (marine types being particularly active in this respect) to histamine (Geiger, 1948), and several cases of food poisoning, after the ingestion of mackerel, sardine and tunny, have been attributed to the high histamine content of the fish eaten (Markoff, 1939 ; Strøm & Lindberg, 1945; Legroux, Levaditi, Boudin & Bovet, 1946 ; Legroux & Levaditi, 1947).

The functions of these compounds in muscle physiology are not very clear. Yudaev (1949) seems to have established that in mammalian (rabbit) muscle, carnosine and anserine are absent in the early embryonic stages of growth and only appear with the formation of muscle function. Increased load on the muscle leads to increases in both carnosine and anserine, whilst denervation with loss of mobility causes carnosine to disappear and anserine to be greatly reduced in amount. Bate-Smith (1938) has pointed out that about 75% of the buffering capacity of mammalian skeletal muscle in rigor between pH 7·5 and 5·5 is due to the non-protein extractives, and about half of this buffering is due to carnosine and anserine, the maximum capacity being at about pH 7·0.

As a final note on the imidazole derivatives in fish, it may be of interest to mention the recent isolation by German workers (Ackermann & Müller, 1941) of spinacin from dogfish liver, as this is the first naturally occurring representative of the imidazole-pyridine condensed ring system.

IV. MISCELLANEOUS GROUPS

Urea. Much data exist on the distribution of urea in the muscle and body fluids of some fish, in particular, the marine elasmobranchs. This is largely due to the fact that urea retention in man and other animals is usually associated with pathological conditions, but is a normal physiological process in elasmobranchs, and hence of considerable biological interest.

It is not intended to give here all the data on the distribution of urea in fish and crustacean muscle, as this has been adequately reviewed by Smith (1936) (see also Fearon, 1926); representative examples only are given in Table 9. It will be noted there that urea is present in the skeletal muscle and all body fluids of the elasmobranchs in quantities amounting on the average to 2,000 mg. %. It is present in much lower quantities in freshwater elasmobranchs (650 mg. % in the blood and probably in similar quantities in the flesh), and is present in traces only in freshwater and marine teleosts and crustaceans and molluscs. According to Smith (1936), urea is retained by the elasmobranchs because of the relative impermeability of the gills and integument to it and because it is actively conserved by the elasmobranch kidney. In these fish it almost certainly plays an important rôle in osmotic regulation, or at least completely alters their osmotic relations as compared with teleost fishes.

To summarize the nature and extent of the distribution of the extractives mentioned above in very fresh fish, it might be useful at this point to examine how far the total non-protein nitrogen can be accounted for by these groups, in three typical examples of fish and one crustacean. Apart from Campbell's (1935) and Jakobsen & Mathiesen's (1946) data, the figures (Table 10) have been calculated from average values of the various constituents in given species. Table 10 shows that in the fish some 73 to 95% of the non-protein nitrogen can be accounted for, but only about 30% in lobster. In cod, about 20% is present as trimethylamine oxide, 44% as creatine, 10% as carnosine and volatile base, 88% in all. In skate, over 50% is

Table 9. *Urea content of fish and shellfish*

Species	Muscle in mg. %	Blood in mg. %	Liver in mg. %	Author
MARINE ELASMOBRANCHS :				
Dogfish (*Scyllium canicula*)	1820–2160 (1950)	2360–2710 (2610)	1010–1890 (1360)	Schroeder (1890)
,, ,,	2130–2160 (2150)	2240–2340 (2290)		Kisch (1930)
,, ,,		2670–2760		Baglioni (1916)
Skate (*Raja stabuliformis*)	800			Campbell (1935)
Electric ray (*Torpedo galvani*)	1920	1940		Baglioni (1916)
Electric ray (*Torpedo ocellata*)	1530–1760 (1620)	1500–1770 (1640)		Kisch (1930)
Sting ray (*Trygon violacea*)		2840		
Shark (*Sphyrna zygaena*)	1820	1850		
FRESHWATER ELASMOBRANCHS :				
Pristis microdon	about 650	650		Smith (1931)
MARINE TELEOSTS :				
Haddock (*Melanogrammus aeglefinus*)	1·98			Campbell (1935)
,, ,,	14·5			Komarov (1933)
Cod (*Gadus callarias*)	2·3			Campbell (1935)
Salmon (*Salmo salar*)	1·3			
Herring (*Clupea harengus*)	2·9			
Tunny (*Germo germo*)	2·3			Noguchi (1932)
Sea bream (*Sparus* sp.)	2·6			
Sardine (*Sardina melanosticta*)	0·45			
Dab (*Limanda* sp.)	9·91			
SHELLFISH :				
Lobster (*Homarus americanus*)	0·07			Campbell (1935)
Crab (*Portunus trituberculatus*)	0			Noguchi (1932)

accounted for as urea, the rest being creatine, trimethylamine oxide and volatile bases—77% in all. In herring, 50% occurs as creatine, 14% trimethylamine oxide and 9% as volatile bases.

Table 10. *Distribution of non-protein nitrogen in fish and crustacean muscle (expressed as % of total non-protein nitrogen)*

Nitrogen in form of	Cod		Skate	Herring			Lobster
Trimethylamine oxide	20·0	38·0*	6·0	33·0*	14·0	15·0	22·0*
Betaine	3·0		2·0		?	?	
Creatine	44·0	39·0	9·0	42·0	50·0	60·0	1·0
Carnosine	—	?	—	—	—	5·10	?
Anserine	10·0	?	—	—	—		
Histidine	—	?	?	?	?	—	—
Urea	0·3	0·4	51·0	1·0	0·3	?	0·4
Total volatile bases (mainly NH_3)	11·0	2·0	9·0	4·0	9·0	10·0	3·0
Total % accounted for	88·3	79·4‡	77·0	80·0	73·3‡	95·0†§	26·4‡

* Lysine fraction (mainly betaine and trimethylamine oxide).
† Alcohol soluble fraction of the muscle.
‡ After Campbell (1935)
§ Jakobsen & Mathiesen (1946).

V. VOLATILE BASES IN STALING FISH

It was stated at the outset that in very fresh fish the volatile base fraction was small in amount and consisted almost wholly of ammonia. As fish spoil, however, considerable changes can occur in both the nature and amount of this fraction in the flesh (Shewan, 1937, 1948, 1949). This can best be illustrated by taking three typical examples from the main groups of fishes ; viz. haddock (gadoids), dogfish (elasmobranchs) and perch (freshwater fish). Fig. 3 shows the changes occurring in ammonia, di- and trimethylamine, and trimethylamine oxide in haddock during stowage for some thirty-five days in ice. It will be seen that whilst trimethylamine oxide falls rapidly, all the bases including ammonia show considerable increases. At the end of fifteen days, at which point the fish are no longer edible, trimethylamine oxide has fallen from 65 mg. % to 35 mg. %, trimethylamine has risen from 0 to 15 mg. %, NH_3 from 10 to 15 mg. % and dimethylamine from 0 to 2·5 mg. % (all expressed as nitrogen). The trimethylamine is formed almost wholly at the expense of the trimethylamine oxide, whilst ammonia at this stage is being formed rapidly, probably by the deamination of protein. The precursor of dimethylamine is unknown, but is probably not trimethylamine oxide.

The rate of formation of trimethylamine appears to depend on the species (Fig. 4) being fastest in whiting and slowest in lemon sole (Shewan, 1949), results which accord well with the known keeping qualities of these species.

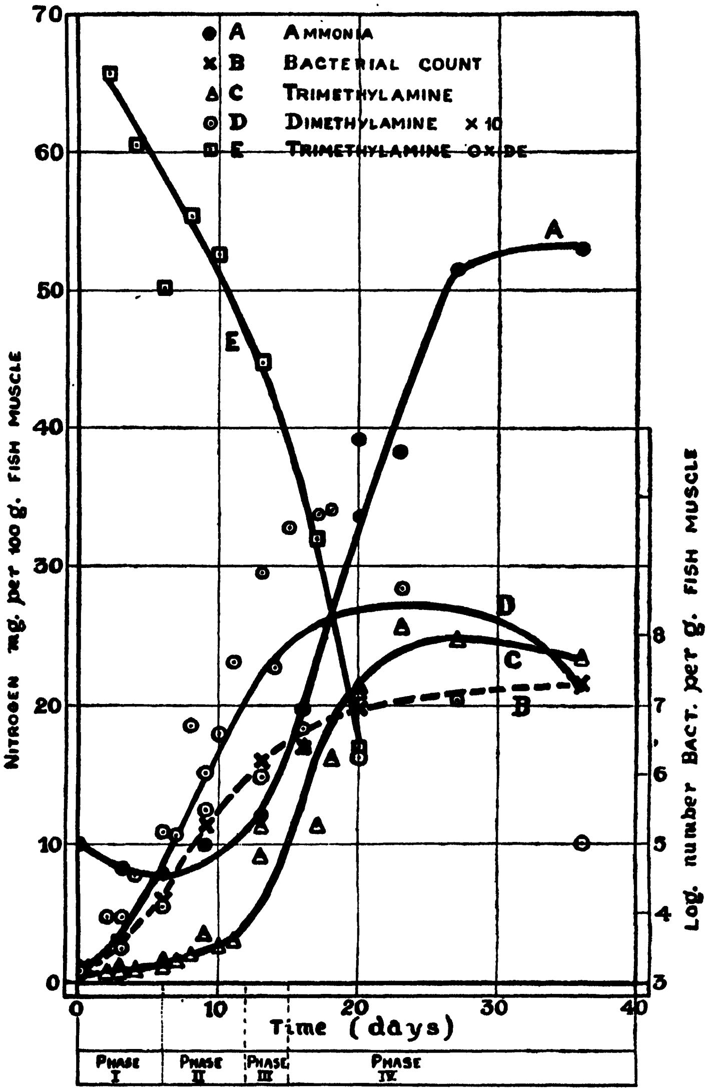


Fig. 3. Variation in the volatile bases, trimethylamine oxide and bacterial counts in haddock muscle during stowage in ice.

In dogfish (Fig. 5) it will be noted that no dimethylamine is formed, presumably due to the absence of the precursor. Moreover, ammonia is formed sooner and more rapidly, due to the bacterial breakdown of urea. Trimethylamine appears to be formed at a similar rate and amount as in cod, despite the larger initial amount of trimethylamine oxide in dogfish compared to haddock. In perch (Table 11) neither dimethylamine nor trimethylamine is formed, due to absence of the appropriate precursors, although ammonia is formed at the same point as in haddock and rises to similar values.

Table 11. *The volatile bases and trimethylamine oxide in the muscles of various species of fish during spoilage in ice.* (*Nitrogen mgms.*% *wet muscle*)

Species	Total volatile base	NH_3	$(CH_3)_2NH$	$(CH_3)_3N$	$(CH_3)_3NO$
	0–7 days				
Dogfish	8·6–8·5	6·0–8·0	0·07–0·07	0·5–1·0	201–290
Haddock	11·3–11·0	10·0–8·0	0·08–0·06	0·56–1·5	46–41
Perch	14·36–10·0	12·57–8·5	Very slight	0·25–Very slight	Very slight
	7–11 days				
Dogfish	8·5–12·0	8·5–10·0	0·07–0·07	1·0–1·2	190–170
Haddock	11·0–19·5	8·0–10·5	0·06–0·14	1·5–6·5	41–29·5
Perch	10·0–9·5	8·5–10·0	Very slight	Very slight	Very slight
	11–15 days				
Dogfish	12·0–45·0	10·0–39·5	0·07–0·07	1·2–6·0	170–145
Haddock	19·5–34·5	10·5–15·5	0·14–0·17	6·5–11·5	29·5–19·0
Perch	9·5–16·0	10·0–15·5	Very slight	Very slight	Very slight
	15–25 or 30 days				
Dogfish	45·0–165·0	39·5–145·0	0·07–0·07	6·0–26·0	145–65 (25 days in ice)
Haddock	34·5–78·0	15·5–49·0	0·17–0·16	11·5–24·0	19·0–0 (30 days in ice)
Perch	16·0–80·0	15·5–80·0	Very slight	Very slight	Very slight (25 days in ice)

In marine fish the formation of di- and trimethylamines during bacterial spoilage has led to the suggestion that the estimation of both these amines in fish muscle might form suitable objective indices of spoilage (Beatty & Gibbons, 1937; Shewan, 1949; Reay & Shewan, 1949). Although some measure of success has been attained, much more will have to be done before the tests could be applied in practice.

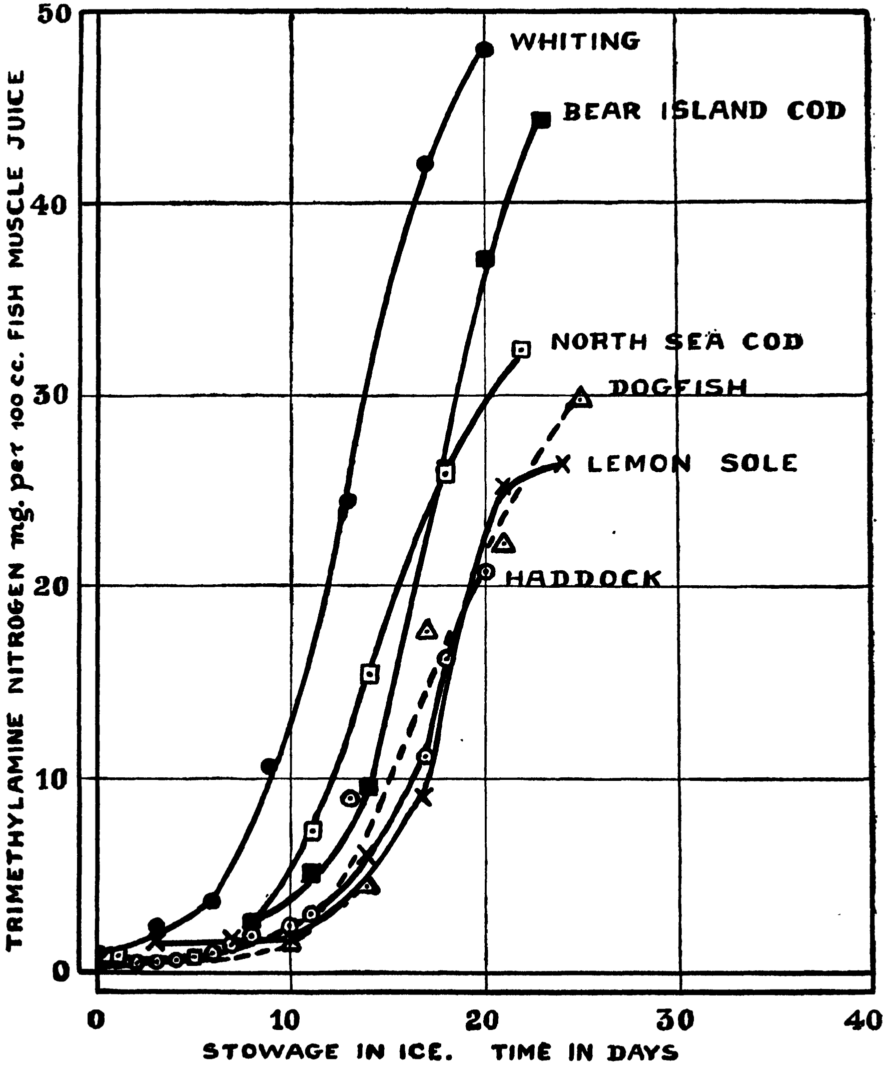


Fig. 4. Variation in the trimethylamine content of muscle of various species of fish stowed in ice.

Apart from trimethylamine oxide, urea and histidine mentioned earlier, the fate of the other extractives during the spoilage of fish is largely unknown.

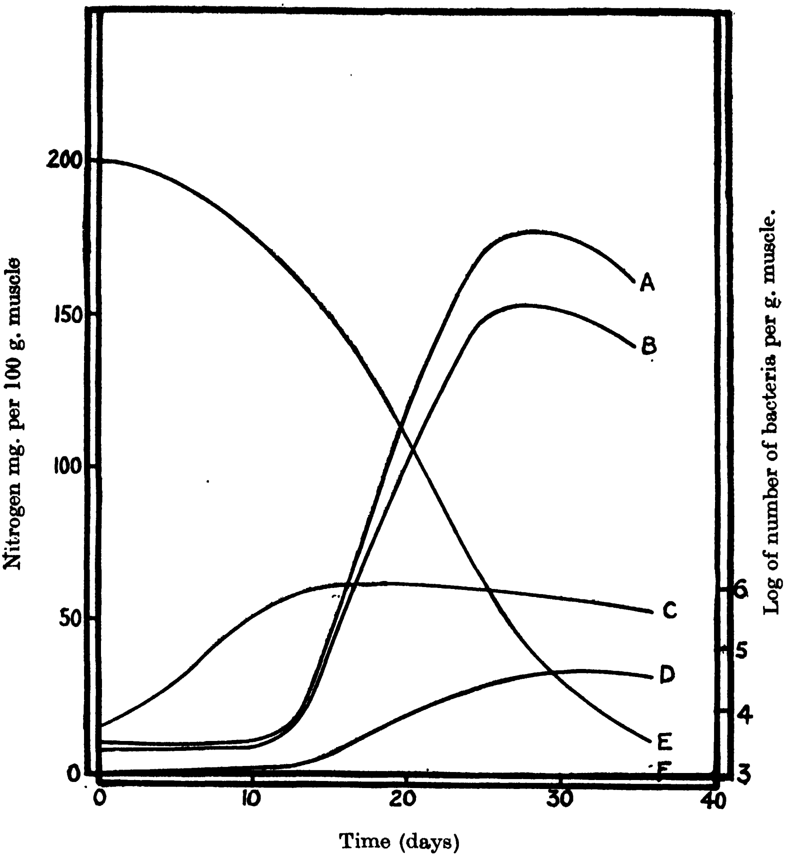


Fig. 5. Variation in volatile bases, trimethylamine oxide and bacterial count in dogfish's muscle during stowage in ice.

Curve A, total volatile bases; B, ammonia; C, bacterial count; D, tertiary amines; E, trimethylamine oxide; F, secondary amines.

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4. THE CHEMISTRY AND METABOLISM OF FATS IN FISH

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The Chemistry of Fish Fats

There are three kinds of chemical knowledge which it is necessary to have about a fat. These are (1) the precise nature of all the various fatty acids to be found in the fat, (2) the quantitative proportions of these different acids, and (3) in what form they are present in the total fat, e.g. what types of glyceride and, sometimes, of esters of other alcohols than glycerol are present and in what proportions. The fats of fish are of unusually complex chemical make-up, even for the class of natural products, the fats, which are, as a whole, almost always composed of a mixture of many ingredients. The knowledge available under each of the three headings given above is still far from complete, especially for the third item, but even so it is far too extensive to do more here than summarize its main features. No attempt will be made to describe the specialized experimental techniques used in this field of chemistry.

Limiting our attention to true fishes, in the zoological sense, the fatty acids present in the depot fats are all of the common straight-chain, monocarboxylic series which is typical of natural fats in general. Only acids containing an even number of carbon atoms are found, and the chain length may range from 12 to 26 or even, perhaps, 28 carbon atoms. While the fat of no single species seems to contain representatives of all this range of chain lengths, fish fats are always characterized by the presence of a large range of homologues, e.g. C_{14}, C_{16}, C_{18}, C_{20} and C_{22} acids, in contrast to the fats of terrestrial species, e.g. plant depot fats, where the fatty acids are frequently restricted to those of 16 and 18 carbon atoms—other derivatives amounting to little more than traces. I shall have more to say later about possible reasons for this complexity of the fats of fish.

For each length of carbon chain, there is almost always more than one acid present, sometimes a mixture of a saturated acid and one or more unsaturated derivatives, sometimes a mixture of unsaturated compounds only. With increasing length of chain, the limit of unsaturation tends to become progressively greater, and the mixtures more complex, as would, in fact, be expected. Thus, for the C_{22} acids, the mixture is well known to contain representatives with one, five and six double bonds (Lovern, 1942), and derivatives with two, three and four double bonds have also been reported (Baudart, 1948). The quantitative separation of mixtures of this kind is not a practicable proposition, and even the qualitative isolation of reasonably pure individual acids for the determination of structure is a laborious task. Such work has further shown that in different species of fish, isomeric forms of the acids exist—e.g. Baudart concluded that the C_{22} pentaethylenic acid from the liver oils of the tunny and a shark was mainly $\Delta^{4,8,12,15,19}$-docosapentenoic acid, but from cod-liver oil mainly the $\Delta^{4,8,12,16,20}$-acid. Sometimes mixtures of such isomers seem to occur even within a species, as Baudart found that, for the C_{20} tetraethylenic acids, a shark-liver oil contained the $\Delta^{6,10,14,18}$-isomer, tunny-liver oil the $\Delta^{4,8,12,16}$-isomer, and cod-liver oil a mixture of both of these. Even qualitative separation of such isomers is out of the question with present knowledge and technique, and the composition of mixtures can only be deduced by an expert of long experience from the products of oxidative breakdown and similar analytical data.

I have deliberately emphasized not only the complexity of the mixture of fatty acids present in the fats of fish, but also the difficulties in the way of their quantitative separation, as a necessary introduction to a brief account of the quantitative composition of these fats. Certain condensations have been found necessary in practice, and usually no attempt is made to express the percentage of, say, C_{20} or C_{22} unsaturated acids, as other than a group, giving at the same time a measure of the mean unsaturation of this group—e.g. —5·5 H would indicate that 5·5 hydrogen atoms are needed to saturate an ' average ' molecule of the mixture, or, alternatively, that the ' average ' molecule contains 2·25 double bonds.

Table 1. *Composition of the mixed fatty acids of cod liver oil* (*wts.* %)

Saturated acids			Unsaturated acids				
C_{14}	C_{16}	C_{18}	C_{14}	C_{16}	C_{18}	C_{20}	C_{22}
4·0	11·0	1·2	0·1 (−2·0 H)	11·1 (−2·0 H)	27·2 (−2·5 H)	26·8 (−5·0 H)	18·6 (−7·2 H)

Cod-liver oil is not only one of the best-known examples of a fish fat, but has probably been analysed more often, and by more workers, than any other fish fat. Since, in addition, it possesses a typical fatty acid composition—approximating, indeed, to the average for all the marine species of fish so far examined (Lovern, 1942)—it can well serve as an illustration of the general quantitative fatty acid composition of the fats of fish (see Table 1). Considerable variations from this composition have been recorded by various workers using samples from different parts of the world, variations up to 100%, in fact, for some of the unsaturated acid groups. There is no reason to doubt the genuineness of these variations, but the analysis given in Table 1 has been chosen partly because it was confirmed by repetition, and partly because that particular specimen did, in fact, approximate so closely to an ' average ' marine fish fat.

It can be seen that the fatty acids are made up of about 16% of saturated acids and 84% unsaturated acids. The former group is mainly palmitic (C_{16}), with smaller amounts of myristic (C_{14}) and even less stearic (C_{18}). The unsaturated acids contain relatively large proportions of C_{18} and C_{20} derivatives, somewhat less of C_{16} and C_{22} derivatives, and only traces of C_{14} acids. The trend towards higher limits of unsaturation, and towards higher average unsaturation, with increasing length of carbon chain is reflected in the mean unsaturation figures for the various groups. Actually, the C_{16} unsaturated acids probably have a mean unsaturation corresponding to about —2·2 H, as shown by later work, but C_{14} acids do not usually contain polyethylenic derivatives—they do so, however, in the fats of certain species, e.g. young salmon (Lovern, 1934 *b*).

The total number of different fatty acids reported to be present in a fat such as cod-liver oil is nineteen—three saturated, five monoethylenic, two diethylenic, three triethylenic, three tetraethylenic, two pentaethylenic and one hexaethylenic (Baudart, 1948). It is now well established, especially by the extensive work of Hilditch and his school, that the fatty acids in natural fats do not occur as simple triglycerides, e.g. tripalmitin or triolein. On the contrary, the rule is rather maximum heterogeneity, with a simple triglyceride only occurring when one acid is present in such overwhelming proportion that it cannot all be distributed in mixed glycerides. It can readily be appreciated, therefore, that with about twenty fatty acids to be assigned in as heterogeneous a manner as possible to the three hydroxyl groups of glycerol, the number of individual mixed triglycerides making up cod-liver oil, or any other fish oil, is enormous. Even if the fat is fully hydrogenated, so that only five acids are present, the glyceride structure of the fat is still extremely complex.

These mixed glycerides have closely similar physical properties, solubilities, etc., and separation is, therefore, always difficult and incomplete. It is quite impossible to give any analytical picture of the glyceride composition of such a complex fat as that of a fish. Harper & Hilditch (1937) and Hilditch & Terleski (1937) have shown experimentally that fish oils do, in fact, follow the same trend as other fats in having their fatty acids spread as widely as possible throughout the various glycerides. Greitemann (1925), working with fully hydrogenated whale oil (analogous chemically to fully hydrogenated fish oil), has actually isolated several of the heterogeneous triglycerides—e.g. myristo-palmito-arachidin (C_{14}–C_{16}–C_{20}) and others —while Suzuki (1931) and his colleagues have isolated the bromo derivatives of many of the mixed triglycerides from brominated fish oils—e.g. hexadeceno-stearidino-clupanodonin (C_{16} monoethylenic–C_{18} tetraethylenic–C_{22} pentaethylenic) and many others have been claimed.

Fatty acids are not always present in fish fats as triglycerides. In the liver oils of certain sharks and related species they are present, at least in large part, as esters of such compounds as chimyl, batyl and selachyl alcohols. These alcohols are actually ethers formed between higher normal fatty alcohols—e.g. hexadecyl, octadecyl and octadecenyl alcohols—and one of the α–hydroxyl groups of a glycerol molecule. They are thus dihydric alcohols, and the esters with two fatty acid radicals are alkoxy-diglycerides having very similar physical properties to the more usual triglycerides.

I have deliberately confined this paper to the fats of true fish. Had I also included the fats of other aquatic animals, e.g. crustaceans and certain marine mammals, the account of both the fatty acid composition and the mode of linkage of these acids in the total fat would have become still more complex.

In all that follows in this paper, whenever I refer to the composition of a fish fat, I shall really be thinking of the composition of the mixed fatty acids, since the glyceride composition is beyond our analytical methods, and would also be beyond the possibility of expression in any table of normal size. For the fatty acids I shall limit the picture to the mode of expression already given for cod-liver oil, i.e. mixtures of unsaturated acids containing the same number of carbon atoms but having different numbers of double bonds will be considered as entities.

The Metabolism of Fats in Fish

Turning from the chemistry of these fats to their metabolism by the fish, the first item for discussion would seem to be the digestion and absorption of fats in fish. The natural diet of many species of fish can be very rich in fat, e.g. when larger fish are intensively feeding on herrings containing about 20% of fat. Fish have been shown to have very marked capacity for digestion and absorption of fat. Thus, McCay, Bing & Dilley (1927) have shown that brook trout can thrive on a diet containing 57% of fat. McCay & Tunison (1934) fround that brook trout maintained at 8° C. could absorb about 90% of administered cotton-seed oil or salmon oil, and from 50–80% or more of a hydrogenated fat ‘ Crisco ’, whether fed at 7 or at 25% of the total diet. It is to be noted that, although the cotton-seed and salmon oils were liquid at 8° C., the hydrogenated fat had a melting-point of 43° C. This ability of fish, in spite of their relatively short and simple alimentary tract, to make good use of fats which are solid at the body temperature of the fish, has also been noted by Lovern (1940), who found efficient absorption and utilization of ethyl palmitate by the eel.

Lipase is very active in the gastro-intestinal tract of fish. Thus, Lovern & Morton (1939) found extremely rapid *post-mortem* production of free fatty acid in the lipids of halibut intestinal tissue, and for various species of fish it proved impossible to extract from washed and still living intestinal tissue lipids with a free fatty acid content of less than 5–10%. In the course of 5–10 minutes *post mortem*, the free fatty acid content of the intestinal lipids had risen to over 20%, and further delay in extraction led to an increasingly high proportion of free fatty acids.

It is by no means certain how important a role lipolysis plays in fat absorption in fish. Greene (1913), in studies on fat absorption in the king salmon, failed to find any structures corresponding to the villi and lacteals of the mammalian intestine. Absorption of fat, whether during natural feeding on a mixed diet, or when olive oil was administered *via* the rectum, could readily be demonstrated histologically to take place through the mucosal epithelial cells of the intestine, the pyloric caeca and the stomach. Absorption was most active in the pyloric caeca, and least, but by no means insignificant, in the stomach. From the epithelial cells the fat droplets passed into the underlying layer of connective tissue, the *tunica propria*, and there they remained for a considerable time after the cessation of active fat absorption. How they gradually disappeared, involving transport through the dense and continuous sheath of the *stratum compactum*, is quite obscure. It could not be as large droplets, the dispersion of which would be a necessary preliminary.

When considerable quantities of fat different in composition from the normal depot fat of the fish are ingested, the direct deposition of some of this in the depots can readily be demonstrated, just as in many other species of animals, though not, apparently, in all (Shorland, 1950). Thus, when ethyl palmitate was incorporated in the diet of eels, the palmitic acid content of their depot fat rose from 18 to 26% (Lovern, 1940). When eels were fed exclusively on herring, the final depot fat of the eels corresponded almost exactly to a definite mixture of original eel fat and herring fat (Lovern, 1938 *b*). When herring feed intensively on the plankton crustacean *Calanus finmarchicus*, their depot fat alters in the direction of a mixture of the original herring fat and *Calanus* fat (Lovern, 1938 *a*). Many similar items could be quoted, but I have selected these, since they have a quantitative basis which is only possible when all the fats in question have been submitted to detailed analysis of the mixed fatty acids. If reliance is placed solely on such figures as the iodine and saponification values, quite misleading results may be obtained, not only because of the complex mixture of fatty acids which is involved, but also because of the occurrence of enzymic systems in the fish which can sometimes appreciably modify the depot fat—e.g. by hydrogenation or dehydrogenation.

The extent to which dietary fat may modify the depot fat is not solely dependent on the level at which it is fed and the duration of the feeding period. It is at least as much dependent on the general metabolic level of the fish and its rate of expenditure of muscular energy. This is well illustrated by the experiments already mentioned in which eels were fed exclusively on herrings. One group of twenty-five fish was maintained at 14° C. and another similar group at 23° C., in each case for six months. In the warmer water the fish were much more active and consumed a total of nearly 6 kg. of herrings, while in the colder water they tended to be sluggish and only consumed some 4 kg. of herrings. Weight increase in the warm water was about 26% and in the cold water only about 16%, but the final fat of the former corresponded to a mixture of 78% original eel fat and 22% of herring fat, whereas the fat of the latter corresponded to a mixture of 62% original eel fat and 38% herring fat. The results are given in Table 2, expressing the fatty acids as molecular groups, each of the same number of carbon atoms, to avoid the disturbing effect of hydrogenation and dehydrogenation processes in the fish. It may be noted that the results suggest that only part of the ingested fat ever enters the fat depots, and are not in agreement with the concept of a complete turnover between dietary and depot fat. On the latter view, the writer calculated that in the cold-water experiment the final fat would have corresponded to a mixture of only 28% original eel fat and 72% herring fat, and in the warm water to a mixture of 24% original eel fat and 76% herring fat—figures almost the reverse of what was actually found.

Table 2. *Fatty acid composition of the depot fat of eels fed on herring* (*wts.* %)

Material	C_{14}	C_{16}	C_{18}	C_{20}	C_{22}
Eels kept at 14° C.	6·4	24·0	33·2	22·2	14·2
62% eel fat plus 38% herring fat	6·0(+)	23·8	33·0	23·2	14·0
Eels kept at 23° C.	6·6	24·8	35·0	22·6	11·0
78% eel fat plus 22% herring fat	5·3	25·1	36·0	21·9	11·7
Original eel fat	4·3	27·0	40·1	20·1	8·5
Herring fat	8·8	18·5	21·3	28·3	23·1

With most species of fish, fat synthesis from carbohydrate will not confuse the picture, as so often happens with herbivorous or omnivorous land animals. However, some fish do feed exclusively on vegetable diets rich in carbohydrate and extremely poor in fat—e.g. certain tropical carp artificially raised for food—and it has been established experimentally that brook trout, for instance, can readily digest, absorb and utilize starch, dextrin and sucrose (Tunison, Phillips, McCay, Mitchell & Rodgers, 1939), although it is not known whether these compounds can be converted into fat. The tropical carp referred to, fed exclusively on grass, had fat appreciably different from that of European carp feeding on a mixed natural diet (Lovern, 1935 *b*)—containing considerably more C_{18} acids and less of the typically 'fish oil' acids—hexadecenoic and C_{20} unsaturated acids (C_{22} acids seem to be entirely absent from all carp fats).

This observation of the deposition of a fat with less strongly marked 'fishy' characteristics when the diet is of a non-fishy nature raises the question as to how far the peculiarly complex mixture of fatty acids characteristic of the fats of most fish is due to their regular ingestion of food fat of such a type. Synthesis from carbohydrate is ruled out, and synthesis from ingested protein is unlikely to be significant, since fish, unlike mammals, continue to grow throughout life and increased protein intake results in an increased growth rate. Dietary fat would thus seem, of necessity, to be the ultimate source of the depot fat of most fish—whether selectively modified by the fish or not.

There is one piece of evidence which seems strongly to support the view that the general complex chemical pattern of the fats of fish is due to exclusive ingestion of fat of just such a nature. In marine species on the one hand, and freshwater species on the other, we find two distinct types of fat (Lovern, 1937). True, variations of a specific nature may be superimposed on these types, but the types themselves can always be detected. Species which live in estuarine waters, or which regularly visit both environments, have an intermediate type of fat. Table 3 illustrates the nature and extent of the differences in these three types, which are mainly confined to the unsaturated acids, by means of average compositions derived from a large number of species. The boundary values represent the cross-over points on frequency distribution curves, and give an idea of what is actually found in estuarine and euryhaline species (Lovern, 1942).

Table 3. *Average compositions of the fats of fish* (*wts.* %)

Type	Total C_{16}	Unsat. C_{16}	C_{18}	C_{20}	C_{22}
Freshwater	40	20	40	13	2·5
Boundary	30	15	35	17·5	9
Marine	25	10	25	25	15

These type differences between the fats of freshwater and marine fish respectively can also be found in the fats of the plankton crustaceans from the two environments (Lovern, 1935 *a*) and, since these small vegetable-feeding organisms are the main food link between the aquatic plant and animal kingdoms, it is clear that simple deposition of ingested fat at each stage of the chain could account for the observed composition of the fats of marine and freshwater fish. It may be added that when a euryhaline fish, such as the freshwater eel, is kept on the same diet in sea water or in fresh water respectively, no difference can be observed in the fat, but if kept in one environment (e.g. fresh water) and fed on food pertaining to the other environment (e.g. herrings), the fat of the eel is changed in the direction of the second environment (Lovern, 1938 *b*).

Notwithstanding what has been said in the previous paragraphs about the effect of dietary fat, this is certainly not the whole story of depot fat composition in many cases. Again and again instances are encountered where the fish has altered the ingested fat in a selective manner, peculiar to that species or family of species, either before or after deposition in the depots. Nothing is known of the actual site of such transformations. It is interesting to note that it is the depot fat—i.e. reserve fuel or insulating material—and not the structural lipids—phosphatides, etc.—which so often show these specific features of chemical composition. Over the whole realms of plant life and animal life respectively, it is possible to show, in spite of many gaps in the field, that there is a gradual and progressive change, namely a simplification, in the mixture of fatty acids of the depot fats as evolutionary development proceeds (Hilditch & Lovern, 1936). The tendency is towards an increasing preponderance of acids of 16 and 18 carbon atoms, mainly palmitic and oleic acid respectively. However, superimposed on the general picture, in the case of animals, are the effects of specialized diets, e.g. many marine mammals and sea birds have depot fats closely resembling those of fish (Lovern, 1942). Also superimposed on this general picture are variations peculiar to small groups of closely related species, and it is some examples of this type of effect I want to mention now.

Many cases could be cited where there is evidence that a fish can selectively hydrogenate or dehydrogenate one or more of the fatty acids entering into its depot fat. Indeed, mechanisms of this nature had been suggested (Lovern, 1942) from detailed analyses of depot fats before the ability of animals to effect such changes had been so elegantly demonstrated with fats containing deuterium (Rittenberg & Schoenheimer, 1937; Schoenheimer & Rittenberg, 1936). For present purposes I have selected two examples, the herring and certain sharks.

At the end of its winter fast, the herring has almost depleted its fat stores, and about the end of May it commences to feed voraciously on small plankton crustaceans, which are quite rich in fat. The fat content of the herring rises rapidly from 3% or less to over 20%. A main ingredient in the diet is the organism *Calanus finmarchicus*, the fat of which has been analysed in detail (Lovern, 1935 *a*). After about a month, the rate of feeding is markedly reduced, and the fat content of the herring gradually falls, slowly during the summer, and more rapidly with the onset of spawning and of winter. It should also be pointed out that during the month of rapid fat storage, the fat is not incorporated so firmly in the depots as it becomes later in the summer, being largely held quite loosely below the skin. This overloading, so to say, of the fat assimilatory mechanism of the fish is reflected in an overloading of the enzyme systems responsible for certain specific features of herring depot fat. In Table 4 are given the detailed compositions of the unsaturated acids of herring fat at various seasons and also the composition of the unsaturated acids of the fat of *Calanus finmarchicus*. To simplify the table, I have omitted the saturated acids, and also the traces of unsaturated C_{14} acids, which do not affect present considerations—they make up the total to 100% in each analysis given.

Table 4. *Unsaturated fatty acids of herring and* Calanus *fats* (*wts. % of total fatty acids*)

Sample	C_{16}	C_{18}	C_{20}	C_{22}
April, 8% fat	4·6 (−2·6 H)	22·2 (−2·9 H)	22·0 (−3·9 H)	27·3 (−4·2 H)
June, 11% fat	7·5 (−2·7 H)	21·1 (−3·3 H)	27·3 (−4·8 H)	19·5 (−5·7 H)
June, 16% fat	7·0 (−3·0 H)	21·1 (−4·8 H)	30·0 (−5·2 H)	21·2 (−4·8 H)
July, 21% fat	6·4 (−3·4 H)	21·0 (−4·5 H)	28·3 (−5·5 H)	23·1 (−4·6 H)
October, 19% fat	4·9 (−2·7 H)	20·7 (−4·2 H)	30·1 (−4·6 H)	23·2 (−4·3 H)
October, 12% fat	4·9 (−2·8 H)	16·3 (−3·6 H)	28·7 (−4·4 H)	29·1 (−4·1 H)
Calanus finmarchicus	11·8 (−2·4 H)	16·8 (−5·1 H)	23·5 (−7·8 H)	25·1 (−8·1 H)

The first sample may be taken as representing the position in the herring about the end of the winter fast, long after the influence of dietary fat has been withdrawn and, therefore, exhibiting any specific features at their clearest. Actually, more than one type of specific feature is exhibited here, but for present purposes we may note: (*a*) the high degree of unsaturation of the C_{16} acids compared with the much more usual value of about −2·2 H, and (*b*) the low degree of the unsaturation of the C_{20} and C_{22} acids, compared with the usual values of about —6 H and —8 H respectively. The C_{18} acids possess about average unsaturation for a fish fat.

The next three samples cover the period of rapid fat ingestion, and it will be noted that this ingested fat contains C_{16} acids slightly less highly unsaturated, C_{18}, C_{20} and C_{22} acids markedly more unsaturated than those of the fish. The herring appears still further to exaggerate the high unsaturation of the C_{16} acids, in spite of dietary acids tending in the reverse direction. C_{18}, C_{20} and C_{22} acids at first follow the direction of the dietary fat, but C_{22} acids and (later) C_{18} acids subsequently begin to be modified faster than they are ingested. It is as though the enzyme systems responsible, at first overwhelmed, progressively increase in activity.

The two October samples, the second showing the fall in fat content due to spawning, represent the state of affairs long after the intensive influence of dietary fat has been removed, and they should, of course, be considered as forming a cycle leading back to the first sample. During the phase represented, then, by these three samples, C_{16} acids fall in unsaturation—i.e. the initial dehydrogenation is replaced by hydrogenation. C_{20} acids, the last of the other groups to be modified away from the direction of the diet, are progressively hydrogenated, and further hydrogenation of the C_{18} and C_{22} acids occurs.

A point I want to stress is the apparent independent action of these hydrogenation or dehydrogenation enzyme systems on the different groups of fatty acids. In the second sample we appear to have dehydrogenation of C_{16} acids alone. In the next stage, we have simultaneously hydrogenation of the C_{22} acids. Next we get simultaneous dehydrogenation of C_{16} acids and hydrogenation of C_{18} and C_{22} acids. Finally, we get simultaneous hydrogenation in all groups, involving a complete reversal of activity in the case of C_{16}. It could, of course, be argued that selective utilization of the more unsaturated derivatives is an alternative explanation of the falls in degrees of unsaturation, but extensive data show no good evidence that selective utilization of this type ever occurs, and also show many other examples of dehydrogenation or hydrogenation processes, often specifically confined to one or more of the component acid groups, e.g. in the developing eggs of the salmon (Lovern, 1936). The author has elsewhere discussed these findings in more detail (Lovern, 1942).

Some of the most striking examples of probable hydrogenation of ingested fat are to be found in certain sharks, where the unsaturation of each group of acids is markedly reduced, in some cases leaving no polyethylenic acids at all, even in the C_{20} and C_{22} acid groups, e.g. in *Scymnorhinus lichia* (Hilditch & Houlbrooke, 1928). Simultaneously, small amounts of higher saturated acids, arachidic and behenic acids, not normally present in fish fats, make their appearance. In these particular sharks, moreover, yet another evidence of hydrogenation processes is to be found, namely the production on a major scale of higher fatty alcohols, combined as ethers with glycerol. The inter-convertibility of fatty acids and fatty alcohols in the rat has been shown with the aid of deuterium (Stetten & Schoenheimer, 1940 *a*, *b*). It is surely significant that all these striking signs of fat hydrogenation occur together, whereas in certain other species of sharks none of them is found, e.g. in the thresher shark, *Alopoecia vulpes* (Lovern, 1930).

There is another way in which certain fish can specifically modify their ingested fat. They may alter the chain length of one or more of the fatty acids, either increasing or decreasing it. The most striking instances of chain shortening are not found in fish, but in the toothed whales (Lovern, 1942). However, some good illustrations of chain lengthening can be found among the liver fats of sharks and related fishes, where simultaneously with markedly reduced proportions of C_{14} and C_{16} acids, considerable amounts of C_{24} acids appear. In most fish fats C_{24} acids are either absent or present in little more than traces. Coupled with this shift at the two ends of the series of constituent acids are found disturbed proportions of the intermediate groups, C_{18}, C_{20} and C_{22}. In most fish fats the acids form a rather smooth series, with C_{16}, C_{18} and C_{20} acids roughly equal in amount and the proportions of C_{14} and C_{22} acids tailing off. The different picture with these particular sharks can be seen from Table 5, in which all fatty acids of any particular chain length are grouped together, in view of the known presence of fat hydrogenation processes. The normal picture is shown by the ‘ average ’ marine fish, and many species of sharks conform fairly well to this average. *Squalus acanthias* comes very near to it, but C_{24} acids are introduced at the expense of C_{16}. In *Chimaera monstrosa* (the ratfish, not a shark, but closely related) there are many irregularities. C_{14} acids disappear, C_{16} are markedly reduced, a little C_{24} appears, and enormous amounts of C_{18} acids are found. *Scymnorhinus lichia* has a break in the even sequence, in that C_{20} acids are less than C_{22} acids and, in both this specimen and in the mixed one, C_{14} and C_{16} acids are reduced and considerable amounts of C_{24} acids appear.

Table 5. *Fatty acids of certain sharks and related species* (*wts.* %)

Species	C_{14}	C_{16}	C_{18}	C_{20}	C_{22}	C_{24}
Squalus acanthias	6·0	19·5	27·5	29·0	12·0	6·0
Chimaera monstrosa	Nil	10·9	57·8	20·9	8·3	2·1
Scymnorhinus lichia	1·6	18·3	32·7	11·4	26·0	10·0
Mixed sp.	1·2	16·7	36·7	17·6	15·8	12·0
Average marine fish	7·0	26·0	27·0	25·0	15·0	Nil

Considering the picture as a whole, one is left not so much with the impression of C_{14} and C_{16} acids being converted into C_{24} acids, as of a progressive shift, two carbon atoms at a time, right along the whole series of acids. Inequalities in the extent of this shift could explain the increase or reduction in the amounts of some of the acids of intermediate chain length.

Sharks are not the only fish to show such effects. Another example is the catfish (Lovern, 1937), *Anarrhicas lupus*, the liver fat of which contains only 1·5% of C_{14} acids, 51·3% of C_{18} acids and 1·9% of C_{14} acids as major evidences of this type of shift, and also contains reduced amounts of C_{20} and C_{22} acids—12·0 and 5·9% respectively. There almost seems to be evidence here of simultaneous chain lengthening (from C_{14} and C_{24} percentages) and chain shortening (from C_{18}, C_{20} and C_{22} percentages). It may be noted that, in the case of rats, feeding of fats containing deuterium has demonstrated the ability of the animals both to shorten and to lengthen the chains by two carbon atoms at a time (Schoenheimer & Rittenburg, 1937 ; Stetten & Schoenheimer 1940 *a*, *b*).

Table 6. *Fatty acids of different depot fats of certain fish* (*wts.* %)

Species	Depot	C_{14}	C_{16}	C_{18}	C_{20}	C_{22}
Conger eel (*Conger vulgaris*)	Liver	5·6	37·2	37·6	12·4	6·9
,, ,, ,, ,,	Peritoneum	1·8	25·0	41·5	18·3	13·4
Halibut (*Hippoglossus hippoglossus*)	Liver	3·9	33·8	34·9	13·8	13·6
,, ,, ,,	Flesh	4·0	21·3	24·5	26·9	23·3
Turbot (*Rhombus maximus*)	Liver	9·1	36·3	27·9	14·0	12·7
,, ,, ,,	Flesh	3·7	24·0	23·8	26·6	21·9

Certain fish also appear to modify their depot fat by distributing it in a selective manner between several depots. This mechanism may operate in addition to modification of ingested fat by one or more of the methods already discussed, or it may be the sole evidence of modification which the species appears to exhibit. Outstanding examples of selective distribution combined with extensive modification, both of chain length and of unsaturation of the total fat of the animal, are furnished by the toothed whales (Lovern, 1942). Among fishes, good examples of selective distribution, but with little evidence of modification of the total fat, are given by the conger eel, the halibut and the turbot (Lovern, 1942) (the last two being related species). Particulars of the depot fats in question are given in Table 6, saturated and unsaturated acids of the same number of carbon atoms being grouped together for present purposes. It will be seen that the differences are in general progressive with molecular size (apart from an erratic result for C_{14} acids for the halibut), the liver depot (which is a subsidiary depot in these species) showing a preference for acids of shorter chain length. Selective distribution in the toothed whales is also progressive with chain length.

The only clue as to how an animal distributes its fat on such a basis is that in all cases so far noted it is the subsidiary depots which contain smaller proportions of longer chain acids, and Lovern (1942) has tentatively proposed the idea of something like a molecular sieve. In the concluding section of this paper, I want to consider the mobilization of depot fat in the fasting fish, and it is interesting, but probably not relevant to the foregoing section, to note that the only evidence of selectivity displayed on mobilization is also concerned with length of chain.

Many fish undergo marked seasonal depletion of the fat depots, and in a few cases detailed analyses have been made of the fat at various stages of the process. It is difficult to interpret some of the results, e.g. in the herring, in view of the activity of enzymic hydrogenation/dehydrogenation systems, but in general it may be said that there is no good evidence in any case of preferential utilization of the more unsaturated constituents. There is sometimes, but not always (e.g. in the salmon, but not in the herring), evidence of preferential utilization of acids of shorter chain length. Both these findings have been paralleled in experiments on rats (Longenecker, 1939). Some results on salmon (Lovern, 1934 *a*) are given in Table 7, the analysis in each case being of the fat of a single fish, which may account for certain erratic features. In previous tables the analyses have been of mixed samples, often including a great many individual fish. Male and female fish are given separately, beginning in each case with a 'fresh run' fish, i.e. one which has just newly entered the river from the sea, and only just begun to fast. For the male series the preferential mobilization of lower acids is particularly marked in the C_{16} series, and the accumulation of higher acids in the C_{22} series. For the female series, corresponding changes are evident in the C_{14} and C_{20} series, with minor parallel changes in other groups. C_{22} acids are out of sequence and there is some evidence suggesting that this may be due to specific transfer of such acids to the developing gonads, a transfer which does not occur in the male fish (Lovern, 1936). The failure of C_{14} acids to fall below about 2% (e.g. in the male series) may be due to the limiting effect of the law of mass action.

Table 7. *Fatty acids of the fat of starving salmon* (*wts.* %)

Fish	C_{14}	C_{16}	C_{18}	C_{20}	C_{22}
Male, 13·9% fat	3·9	25·6	30·8	23·5	16·2
Male, 6·5% fat	2·0	20·3	28·0	26·9	22·8
Male, 1·1% fat	2·3	17·5	28·3	25·8	26·1
Female, 13·2% fat	5·5	20·4	26·8	26·5	20·8
Female, 7·3% fat	2·2	20·2	28·7	28·4	20·5
Female, 3·5% fat	1·9	17·8	30·3	33·2	16·8

One may perhaps briefly summarize the substance of this paper by saying that although fish lay down a depot fat of more complex fatty acid composition than that of many land animals, the enzyme systems which operate to produce this type of fat, or to modify it, are in general normally present in many species of land animals having a simpler type of depot fat, e.g. in rats. The very complexity of fish fats, however, makes it possible to deduce the presence of these enzyme systems by detailed fatty acid analyses, whereas in animals with a simpler type of fat the use of isotopic or other tracer material is often needed. There may be a moral here, and I commend fish to the attention of the biochemist.

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5. CAROTENOIDS IN FISH

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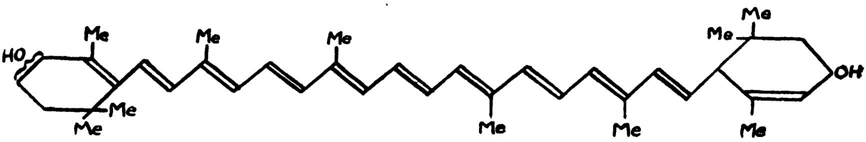
There is a general tendency amongst aquatic animals preferentially to accumulate xanthophylls rather than carotenes, and fish are no exception. In fact, apart from in the ovaries, and perhaps occasionally in the liver, carotenes are completely absent. In the ovaries, only β-carotene is found, and it constitutes a very minor proportion of the total carotenoids. Compared with other classes of aquatic life, xanthophyll distribution in fish is unique in that only three pigments have been unequivocally identified, although a comparatively large number of species has been examined. No differences in pigment distribution have ever been satisfactorily established between marine and freshwater fish and, as it appears that no differences do exist, they are here considered together.

The three xanthophylls occurring in fish are lutein, a pigment very probably taraxanthin, although not yet completely identified, and astaxanthin. The report that zeaxanthin and violaxanthin are present in some species (Goodrich, Hill & Arrick, 1941) has not yet been confirmed. Lutein, the most abundant Phanerogam xanthophyll, has the structure 3 (or 4) : 3′-dihydroxy-α-carotene* ; taraxanthin, first isolated from the petals of *Taraxacum officinale* (Kuhn & Lederer, 1931), is of undetermined structure but contains two rings and four hydroxyl groups ; spectroscopic and biochemical evidence (see later) is consistent with it being a β-carotene derivative, possibly 5 : 6, 5′ : 6′-diepoxy-zeaxanthin. Astaxanthin, 3 : 3′-dihydroxy-4 : 4′-diketo-β-carotene (Kuhn & Sörensen, 1938) is known as an 'acidic' carotenoid because of its ability to enolize and form a salt. This salt is, however, very unstable, and in the presence of air astacin (3 : 3′, 4 : 4′-tetraketo-β-carotene) is formed. Astaxanthin was originally isolated as astacin and until Kuhn & Sörensen's (1938) work proved that astacin was an



Lutein

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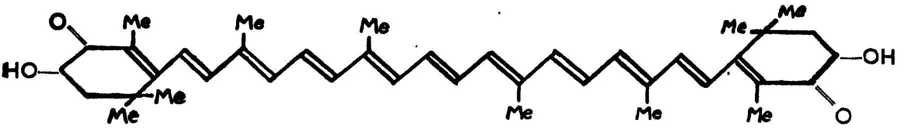
* The usually accepted structure for lutein is 3 : 3′-dihydroxy-α-carotene, but it is possible that 3′ : 4-dihydroxy-α-carotene may be the correct structure (Goodwin & Taha, 1951).

oxidative artefact it was often reported as the naturally occurring pigment. This must be borne in mind when early reports of carotenoids are considered, for it is now generally accepted that only astaxanthin occurs naturally.



Astaxanthin

All the available data concerning the qualitative distribution of carotenoïds in fish are set out in Table 1.

It appears from this table that, in general, fish can be divided into two main classes according to whether they accumulate lutein or taraxanthin, for it is comparatively rare to find both occurring in the same species. Astaxanthin, on the other hand, can be associated with either, but in a very small number of cases occurs alone. It remains to be seen whether these 'astaxanthin-only' species are truly of rare occurrence, or whether the small number encountered is due to the 'sampling' of the species so far examined. The freshwater perch (*Perca fluviatilis*) is specially interesting, for some varieties contain astaxanthin whilst others do not. Those which do not, contain a pigment very similar to astaxanthin, except that it has no acidic properties (Lederer, 1937).

The main repository of fish carotenoids is the skin, where they always occur esterified; considerable amounts are also frequently encountered in the flesh, the ovaries (especially during sexual activity) and the liver; ovarian xanthophylls are always unesterified. Some fish are complete storehouses of carotenoids, for example, Lederer (1937) found considerable amounts of astaxanthin not only in the skin but also in the gills, mouth mucous, iris and sclera of *Beryx dedactylus*. Taraxanthin esters were found in the pigment epithelia and choroid layers of the eyes of *Prionatus carolinus*, *Centropristes striatus* and *Stenotomus chrysops* (Wald, 1936), and esterified lutein was found by Steven (1948) in the posterior hemispheres of the eyes of *Salmo trutta*.

In spite of the large amounts of carotenoids, including β-carotene, which accumulate in the developing ova, there is only one report of carotenoids occurring in spermatozoa; Lönnberg (1935) noted the presence of xanthophylls in the spermatozoa of the pike (*Esox lucius*). No trace of carotenoids has been found in the milt of the sand-eel (*Ammodytes tobianus*) (Goodwin, 1948), or of brown trout (*S. trutta*) (Steven, 1948). Carotenoids were also absent from the brain, spleen, kidney, stomach and intestine of the brown trout (Steven, 1948).

Only two species, *Gadus esmarku* and *Squalus acanthias*, out of the large number examined, have been found to be completely devoid of

Table 1. *Carotenoid distribution in fish*

A. SKIN

Species	Pigments			References
	Lutein	Astaxanthin	Taraxanthin	
Ammodytes lanceolatus			+	Goodwin (1948)
Ammodytes tobianus			+	
Anguilla anguilla	+			Lönnberg (1931)
Aphiga minuta			+	Lönnberg (1938)
Bartus nigrofasciatus		+		Goodwin (1948)
Beryx decadactylus		+		Lederer (1935)
Betta splendens	+ (? also violaxanthin)			Goodrich *et al.* (1941)
Bothus maximus			+	Lönnberg (1938)
Bothus rhombus	+		+	
Callionymus lyra			+	Lönnberg (1931, 1938)
Carassius auratus		+		Lederer (1935)
Caraux trachurus	+			Lönnberg (1938)
Centrolabrus exoletus	+	+	+	Lönnberg (1929, 1938)
Clupea harengus	+		+	Lönnberg (1938)
Colisia fasiata	+ (? also violaxanthin)			Goodrich *et al.* (1941)
Colisia lalia	+ (? also violaxanthin)			
Copeina guttata			+	Goodwin (1948)
Cottus bubalis	+		+	Lönnberg (1931, 1938)
Crenilabrus melops *Crenilabrus suillus*	either taraxanthin or lutein			Lönnberg (1938)
Cyclopterus lumpus			+	
Cymatogaster aggretatus			+	Sumner & Fox (1935 *a, b*)
Esox lucius	(? also lutein 5 : 6-epoxide)		+	Lederer (1938)
Fundulus parvipinnis			+	Sumner & Fox (1933, 1935 *a, b*)
Gadus aeglefinus			+	Lönnberg (1938)
Gadus callarias	+		+	Lönnberg (1929, 1938)
Gadus merlangus			+	
Gadus minutus	+		+	Lönnberg (1931, 1938)
Gadus polachius			+	Lönnberg (1929, 1938)
Gadus virens			+	
Gaidropsarus cimbrius	+			Lönnberg (1938)
Gaidropsarus mustela	+			Lönnberg (1931)
Gasterosteus aculeatus			+	Lönnberg (1938)
Gillichthys mirabilis			+	Sumner & Fox (1935 *a*)
Girella nigricans			+	
Gobius niger	+			Lönnberg (1931)
Hypsypops rubicunda			+	Sumner & Fox (1935 *b*)
Labrus berggylta	+			Lönnberg (1938)
Labrus melops	+			Lönnberg (1931)
Labrus ossifagus	+			Lönnberg (1929, 1938)
Lampetra fluviatilis	+			Steven (1950)
Lampetra planeri	+			
Lota lota			+	Lederer (1935)
Macropodus opercularis	+ (? also zeaxanthin)			Goodrich Hill & Arrick (1941)
Neurophis aequoreus	+			Lönnberg (1931)
Neurophis ophidon			+	Lönnberg (1938)

Table 1 (cont.). *Carotenoid distribution in fish*

Species	Pigments			References
	Lutein	Astaxanthin	Taraxanthin	
Oryzias latipes	+ (? also zeaxanthin)			Goodrich *et al.* (1941)
Perca fluviatilis		+	+	Lederer (1937) Lönnberg (1929)
Pholis gunellus	+		+	Lönnberg (1929, 1938)
Platypoecilus maculatus	+ (? also zeaxanthin)			Goodrich *et al.* (1941)
Pleuronectes flesus	+			Lönnberg (1931)
Pleuronectes kitt	+		+	Lönnberg (1929, 1931, 1938)
Pleuronectes limanda	+		+	Lönnberg (1938)
Raja batis	+			
Raja clavata	+			
Raniceps raninus	+			Lönnberg (1929, 1938)
Salmo gairdneri		+		Baily (1937)
Salmo salar	+	+		Euler & Hellström (1934) Emmerie *et al.* (1934)
Salmo trutta	+	+	(?) +	Lönnberg (1938) Sorenson & Stene (1938) Steven (1948)
Salvelinus spp.		+		Goodwin (1948) Steven (1948)
Scomber scombrus	+		+	Lönnberg (1931, 1938)
Scophthalmus norvegicus			+	
Scorpaena scrofa		(?) +		Krukenberg (1882)
Sebastes marinus		+		Lederer (1938 *b*) Glover (1948)
Siphostoma typhle	+		+	Lönnberg (1938)
Syngnatus acus	+		+	
Trachinus draco			+	
Trigla gurnardus			+	
Xiphophorus helleri	+ (? also zeaxanthin)			Goodrich, Hill & Arrick (1941)
Zoarces viviparus	+		+	Lönnberg (1938)

B. FLESH

Species	Pigments		References
	Lutein	Astaxanthin	
Coregonus albula		+	Wald (1936)
Lophius piscatorus		+	Burkhardt, Heilbron, Jackson & Parry (1934) Sörensen (1934)
Orchorchynchus nerka		+	Baily (1937)
Salmo salar	(? also β-carotene)	+	Euler & Hellström (1934) Emmerie *et al.* (1934) Sörensen (1934) Sörensen & Stene (1938)
Salmo trutta	+	+	Steven (1948)

Table 1 (cont.). *Carotenoid distribution in fish*

C. Ovaries and Ova

Species	Pigments				References
	β-Carotene	Lutein	Taraxanthin-like pigment	Astaxanthin	
Ammodytes tobianus	+			+	Goodwin (1948)
Coregonus albula			+		Euler, Hellström & Klussman (1934)
Eliginus navaga	+ (+3 unidentified xanthophylls)				Lederer (1938)
Esox lucius	+	+			Euler, Gard & Hellström (1932)
Gadus callarias	+	+			
Gadus morrhua	+		+		Eekelen (1934)
Hippoglossus hippoglossus	+	+	+		Euler, Gard & Hellström (1932)
Lota vulgaris	+	+			Lönnberg (1938)
Salmo irideus	+	+	+		Hartmann, Medem, Kuhn & Bielig (1947)
Salmo salar			+		Euler, Hellström & Malmberg (1933) Morton & Rosen (1948)
Salmo trutta	+	+	+		Steven (1948)
Solea vulgaris	+				Euler *et al* (1932)

D. Liver

Species	Pigments				References
	β-Carotene	Lutein	Astaxanthin	Taraxanthin-like pigment	
Cyclopterus lumpus			+		Lönnberg (1938) Schmidt-Nielson, Sörensen & Trumpey (1932)
Esox lucius		+			Euler & Virgin (1932)
Leuciscus rutilus		+		+	Euler & Virgin (1932) Lönnberg (1938)
Lophius piscatorius	(? also lutein epoxide)		+	+	Sörensen (1933, 1934) Lönnberg (1938) Lederer (1935)
Orthagoriscus mola	(? also α-carotene)		+		Sörensen (1933)
Regalescus glesne			+		Sörensen (1934)
Salmo salar		+			Euler & Virgin (1932)
Salmo trutta	+	+			Steven (1948)

carotenoids (Lönnberg, 1929). Perhaps complete absence from these two species should not be accepted without further confirmation, because although in the same report (Lönnberg, 1929) it was stated that carotenoids were also absent from *Raja clavata*, they were later detected in this species (Lönnberg, 1938).

Although qualitative variations between genera and species are small, quantitative variations can be considerable, as evidenced by the data obtained for the ova of two species of trout, the brown (*S. trutta*) (Steven, 1948) and the rainbow trout (*S. irideus*) (Hartmann, Medem, Kuhn & Bielig, 1947) (Table 2). Not only are there marked differences in the total amounts of the pigments present, in confirmation of the early work of MacWalter & Drummond (1935), but there are very marked differences in the relative amounts of the constituent pigments. Table 2 also records all the readily available results of quantitative experiments on fish carotenoids, which, up to the present, have been woefully few. Much remains to be done on this aspect of the subject.

Table 2. *Carotenoid content of fish*

Species	Amount of pigment (μg./g. fresh tissue)		Reference
	Carotene	Xanthophylls	
Ammodytes tobianus			Goodwin (1948)
whole fish ♂	trace	3·6	
whole fish ♀	trace	9·9	
spermatozoa	0	0	
ova	0·3	8·0	
Cymatogaster aggregatus	0	c. 2·7	Young & Fox (1936)
Fundulus parvipinnis	0	1·7–12	Sumner & Fox (1933, 1935 *a*, *b*)
Gillichthys mirabilis	0	4·1–6·2	Sumner & Fox (1933)
Girella nigricans	0	c. 14	Sumner & Fox (1935 *a*, *b*)
Salmo irideus, ova	0·126	0·506	Hartmann *et al.* (1947)
Salmo trutta			Steven (1948)
skin and fins	0	168	
liver	7·5	12	
ovary	3	171	
muscle (red)	trace	36	
eyes (posterior hemispheres)	0	0·2	

FORMATION OF FISH CAROTENOIDS

In common with all other animals, fish do not possess the power to synthesize carotenoids *de novo*. They can, however, alter alimentary carotenoids, as far as present information goes, always by oxidation, and store the resulting products. In this connection the investigations of Sumner & Fox (1933, 1935 *a*, *b*) and Steven (1948) are outstanding. Sumner and Fox divided aquaria-kept killifish (*Fundulus parvipinnis*) into three groups, which were fed, respectively, a xanthophyll (taraxanthin) diet (garibaldi, *Hypsypops rubicunda*), a carotene diet (beach worm, *Thoracophelia mucronata*) and a carotenoid-free diet (flesh of the Californian halibut, *Paralichthys californicus*). On the first two diets the *Fundulus* carotenoids, entirely taraxanthin, increased in both amount and concentration, although the increase on the carotenoid diet was less than on the taraxanthin diet. On the carotenoid-free diet, on the other hand, there was no change in amount but a fall in concentration owing to the growth of the fish. These experiments quite clearly demonstrate that *Fundulus* carotenoids are of dietary origin and that the fish has the power to oxidize carotenes to taraxanthin. This suggests that if β-carotene is the major pigment of *Thoracophelia*, taraxanthin is an oxidation product of β-carotene, and supports the suggestion, made earlier, concerning the structure of taraxanthin. The surf perch (*Cymatogaster aggregatus*), however, does not deal with alimentary carotenoids in quite the same way as does *Fundulus*, for when fed the red shrimp, *Hippolyte californiensis*, which contains β-carotene, neutral (? taraxanthin) and acidic (? astaxanthin) carotenoids, only taraxanthin was absorbed, the β-carotene and astaxanthin being excreted quantitatively. Taraxanthin was hydrolysed in the gut, absorbed, and transported to the skin, where it appeared as the esterified form, esterification having presumably taken place during passage across the gut wall. Excess taraxanthin was stored unesterified in the rectal segment of the gut, whence it rapidly disappeared when the fish was starved. The significance of this storage site is still obscure, but it has been established that the segment does not absorb its pigment from the lumen but obtains it *via* the blood stream, and that it neither excretes taraxanthin into the rectal lumen nor oxidizes it *in situ* (Young & Fox, 1936). Young and Fox do not consider it a temporary storehouse for replenishing dermal carotenoids.

In spite of its failure to absorb astaxanthin, *Cymatogaster* does hydrolyse esters of this pigment during their passage down the intestinal tract. The halibut, similarly, does not appear to store any appreciable quantities of astaxanthin (Glover, 1948), although its main diet is a fish (*Sebastes marinus*) rich in astaxanthin (Lederer, 1937; Glover, 1948); small amounts of astaxanthin have, however, been observed in halibut ova (Euler, Gard & Hellström, 1932).

Work on carotenoid formation in freshwater fish has been almost exclusively confined to trout. Although the pattern of pigmentation in trout is due to genetic factors, there is now no doubt that the lipochrome pigments are of alimentary origin. Davis (1930) claimed that hatchery trout when fed dried salmon eggs (which contain considerable amounts of astaxanthin) showed the same pigment pattern as did wild trout. This was confirmed by Mann (1936), using as food, goldfish, carrots and mixed *Daphnia* spp., although the earlier work of McCay & Tunison (1934) had been somewhat less conclusive. Recently, Steven (1948) has provided quantitative data (Table 3) to demonstrate quite conclusively that trout carotenoids are of alimentary origin. He found that brown trout (*Salmo trutta*), kept in captivity, maintained their natural pigmentation when their diet consisted of the natural diet of a 'balance' tank (permanent flora and fauna of diatoms, filamentous green algae of several species, *Elodea canadensis*, snails, crustacea and insect larvae) supplemented by live Entomostraca (chiefly *Simocephalus*, *Cyclops* and *Daphnia* spp.) and *Corethra* larvae. Analysis of a typical supplement, consisting of 85% of *Daphnia longispina* and the rest mainly *Cyclops* spp., showed that it contained 0·65%, 0·1385% and 0·012% on a dry weight basis of astaxanthin, neutral xanthophylls (mainly lutein) and carotenes, respectively. When the supplement was chopped horse-meat and earthworms, i.e. carotenoid-free, the fish lost their yellow and red pigmentation and their carotenoid content was reduced almost to zero. On transferring these trout to a diet of salmon eggs they rapidly assumed their original pigmentation and their carotenoid content returned almost to that of the controls. Steven attempted to control his diet more strictly by feeding earthworms previously injected with β-carotene, lutein or astacin dissolved in arachis oil, but no increased pigmentation was observed in these cases. The failure was attributed largely to the inability of the trout to deal adequately with the comparatively large amounts of oil in the diet, although Lovern (1950) states that fish absorb fat well. As no carotenoids were observed in the faeces, it appears that the failure may not have been due entirely to non-absorption but possibly to intestinal destruction of the pigments. A further point which should be emphasized is that there is no valid

Table 3. *The effect of diet on the carotenoid content of the brown trout*

Group	Duration on experimental diet	Carotenoid content of skin and fins (μg./g. tissue)	
		Lutein	Astaxanthin
1. Wild trout	—	120	75
2. Aquarium-reared on natural food and carotenoid-rich supplement (see p. 70)	—	90	80
3. Aquarium-reared on natural food and carotenoid-free supplement	9 months	20	0
4. Group (3) transferred to supplement of salmon ova	35 days	80	65
5. Group (3) transferred to supplement of β-carotene	46 days	15	0
6. Group (3) transferred to supplement of lutein	24 days	30	0
7. Group (3) transferred to supplement of astacin	38 days	20	0

reason why fish should be expected to absorb astacin because, as stated earlier, it is not a naturally occurring pigment but an oxidation product of astaxanthin. Steven appreciated this and further suggested similar changes might have occurred during the preparation of the β-carotene and lutein supplements and thus rendered them non-absorbable ; as far as is known, no such changes have ever been observed with these two carotenoids.

There is one unconfirmed report that the (? carotenoid) pigmentation of the pike (*Esox lucius*) is also of alimentary origin.

Although Sumner & Fox's work (1935) showed that *Fundulus* is capable of oxidizing β-carotene to taraxanthin, no confirmation of this in other species has yet been reported. Such an oxidative reaction, either starting from β-carotene or a suitable xanthophyll, must, however, be widespread, for neither phytoplankton nor zooplankton contains taraxanthin (Goodwin, 1951). As astaxanthin is the major carotenoid of zooplankton (Goodwin, 1951), there is no *a priori* reason why most fish should need to form this pigment from other carotenoids. There is, however, nothing to suggest that they do not possess this ability, because such a conversion has been demonstrated in animals such as the locust (Goodwin, 1949).

FUNCTION

(a) *In photo-responses*

The pioneer work of Sumner & Fox (1933, 1935 *a*) and Fox (1936) on marine fish provided strong circumstantial evidence that carotenoids are concerned in photo-responses in fish. Sumner and Fox found that *Fundulus parvipinnis* and *Gillichthys mirabilis* maintained their carotenoid content in different optical environments, although the colours of the fish changed ; it was assumed that the change in colour was due to contraction and expansion of the chromatophores without any quantitative variation in the carotenoid content of the cells. It will be recalled that variations in pigment content of melanophores do generally occur when the degree of contraction alters. *Girella nigricans* reacts to variations in the colour of its environment in a somewhat different manner. In captivity it gradually loses its xanthophylls even on a diet rich in carotenoids ; on a white background, however, pigment loss is much more rapid than on a dark background.

Goodrich, Hill & Arrick (1941) more recently examined the chromatophores of a number of freshwater tropical fish, viz. *Betta splendens*, *Colisia fasiata*, *C. lalia*, *Macropodus opercularis*, *Oryzias latipes*, *Platypoecilus maculatus* and *Xiphophorus helleri*. They found that the xanthophylls lutein and taraxanthin were concentrated in the xanthophores. The erythrophore pigment did not appear to be a carotenoid but a pterin pigment to which Goodrich *et al.* gave the name erythropterin ; apparently no astaxanthin occurred in any of the species examined in this investigation. Certain varieties of *Platypoecilus* and *Xiphophorus* which carry the gene controlling red pigmentation possess a hybrid chromatophore containing both carotenoids and erythropterin; this chromatophore has been termed a xanthoerythrophore.

It remained, however, for Steven (1948, 1949) to provide the quantitative evidence, which quite clearly demonstrated that, in trout at least, lutein and astaxanthin are associated with the xanthophores and erythrophores respectively. Steven first showed that the red spots of the skin contained about twenty times as much astaxanthin as did regions of the skin which contained no red spots. Using a micro-spectrophotometer, he then went on to measure the absorption spectra of single chromatophores (Fig. 1), and found that the xanthophores and erythrophores exhibited spectra corresponding quite closely to lutein and astaxanthin respectively. Further, by a modification of Pantin's (1923) method for measuring inter-cellular pH values, Steven arrived at an estimate of the amounts of pigment in single chromatophores; single xanthophores contained between 11×10^{-6} and 28×10^{-6} μg. of lutein, and single erythrophores between 200×10^{-6} and 340×10^{-6} of astaxanthin. It should be noted that Steven makes no mention of the presence of a pterin pigment in the erythrophores of

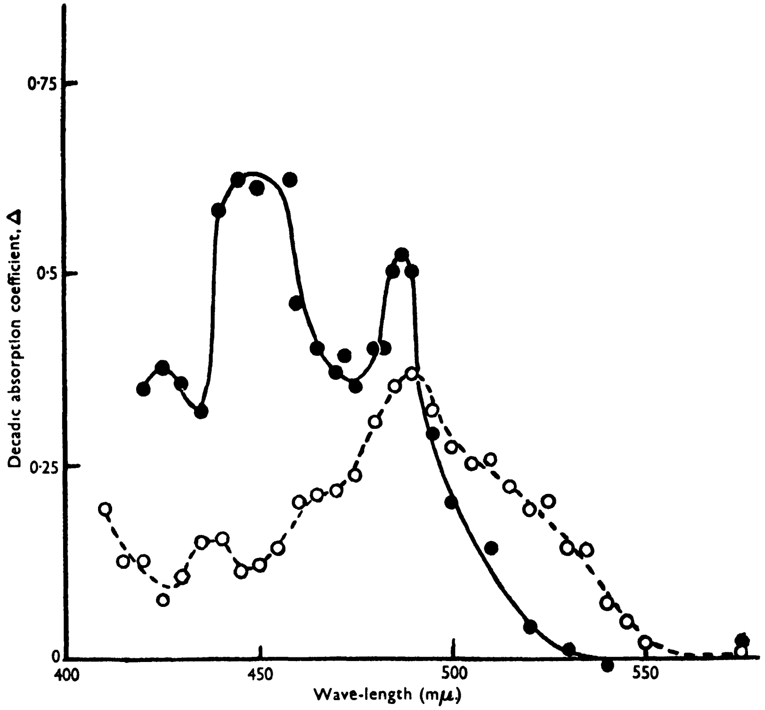
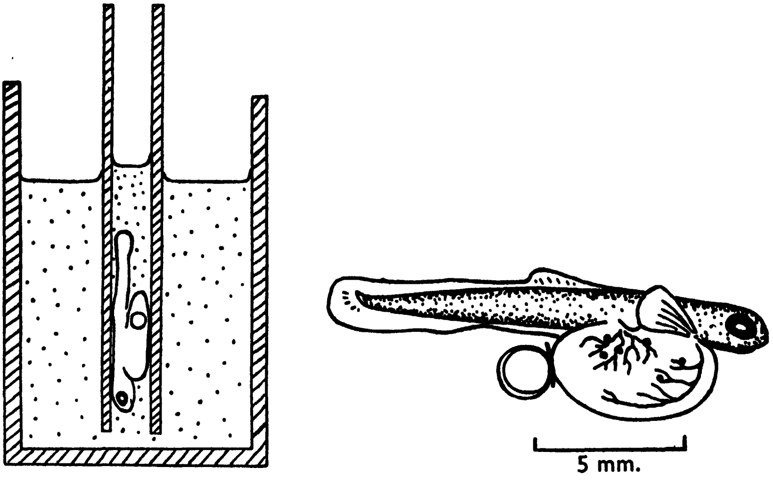


Fig. 1. Absorption spectra of a living xanthophore (continuous line) and a living erythrophore (broken line) (Steven, 1948).

trout. Further evidence of the association of carotenoids with the chromatophores of trout came from dietary studies (Steven, 1948). After 9 months on a carotenoid-free diet, yearling fish exhibited pale yellow xanthophores generally distributed through the skin and fins and a very few orange-pink erythrophores mainly situated at the tip of the adipose fins; the erythrophores which usually accumulate along the lateral line had disappeared. These observations agree with the quantitative studies (Table 3) which showed small amounts of lutein but no astaxanthin in these fish.

Steven's (1949) studies of carotenoid metabolism in the reproductive cycle of the trout also indicated the close relationship between carotenoids and chromatophores. Muscle carotenoids are mobilized into the ovaries during the ripening of the eggs, whilst the skin carotenoids are unaffected. During larval development lutein and astaxanthin are not utilized but are quantitatively transferred in constant ratio from yolk to embryo; during this process they are esterified and accumulate in the skin and fins.



(i) (ii)

Fig. 2. Method of ligaturing the yolk sac of newly hatched trout in order to isolate the oil globules (Steven, 1949).

Using an ingenious experimental technique, Steven removed almost all the carotenoids from the yolk sac of newly hatched brown trout larvae. The larvae were sucked into glass tubes just large enough to hold them and held head downwards in water (Fig. 2, i). After a few minutes in this position the oil globules containing the carotenoids rose to the posterior end of the yolk sac. The larvae were then narcotized with urethane and the oil globule ligatured (Fig. 2, ii). This procedure removed about 90% of the carotenoids from the yolk. Trout treated in this way developed normally on a carotenoid-free diet of the oligochaete worm

Enchytraeus, with the exception that they were very pale in colour and completely lacking in both xanthophores and erythrophores.

When developing larvae of trout from which the yolk carotenoids had not been removed were examined, Steven (1949) found that the rate of transfer of lutein and astaxanthin from yolk to embryo followed more closely the increase in length of the posterior end of the developing embryo than the overall growth measured either as length or weight. As the majority of the carotenoid-containing chromatophores develop in the skin of the posterior end of the body, especially in the tail and adipose fins, this correlation indicates that rate of transfer of pigments is related to the development of the cells which receive them. Steven (1950) has recently observed that lampreys (*Lampetra planeri* and *L. fluviatilis*) contain only xanthophylls, with lutein as the major component, and that these pigments accumulate in the non-expandable lipophores in the dermal and subdermal layers of the skin.

The investigations just described indicate that carotenoids are integral constituents of chromatophores and, as such, are functional in that they are concerned in the photo-responses of fish. Apart from their possible function in reproduction (see below), this is probably the major rôle of carotenoids in fish, because Wald (1943) does not consider their presence in the retinae of fish of great significance, although there exists the possibility that they increase visual acuity by reducing chromatic aberration and glare (Walls & Judd, 1933).

In spite of this now well-authenticated function of carotenoids, a number of interesting observations remain to be explained. For example: (*a*) why do trout on carotenoid-free diets lose their dermal carotenoids, whilst during the reproductive mobilization into the ovaries they are not disturbed, and for what purpose have these carotenoids been utilized ? (*b*) what is the significance of the use of erythropterin and astaxanthin for the same purpose in different species ? and (*c*) what causes the production of ' Silver ' trout observed by Steven (1948) in Loch Leven in which the silver appearance " appeared to be a structural type of pigmentation in the scales or outer layer of the skin, since sections showed that the xanthophores, which lie mostly below the scales in the deeper dermal layers, are as numerous and as well pigmented in Loch Leven as in normal brown trout " ?

(b) *In reproduction*

During the development of the gonads and just prior to spawning, carotenoids are in active movement. Both sexes of *Cyclopterus lumpus* mobilize astaxanthin from the liver into the skin and, probably, the flesh (Schmidt-Nielsen, Sörensen & Trumpey, 1932); similarly, the males of *Fundulus parvipinnis* increase the xanthophyll content of their skin during sexual activity (Sumner & Fox, 1935). This is presumptive evidence that carotenoids mediate in the operation of a secondary sexual characteristic, the change in colour during the spawning season. Proof of such a function has already been obtained with birds (for full references see Goodwin, 1950 *a*).

Mobilization of liver and skin carotenoids into the developing ovaries has been observed in *Fundulus parvipinnis* (Sumner & Fox, 1935) and *Salmo trutta* (Steven, 1948, 1949), and Table 1 indicates that the ova of many species contain carotenoids, although in most of these cases the actual mobilization process has not been investigated. As stated previously, gonadal xanthophylls are, in contrast to those occurring in other organs, unesterified. The presence of free xanthophylls suggests, by analogy with vitamin A, that the pigments are metabolically* functional in the ovaries, because the functional form of vitamin A is probably the free vitamin (but see Goodwin, 1950 *b*), whilst its esters are the storage form. During larval development, however, no detectable utilization of xanthophylls occurs either in the salmon (Morton & Rosen, 1948) or trout (Steven, 1949), they are merely gradually transferred to the embryo, esterified in the process and finally accumulated in the chromatophores. Further, as described in the previous section, removal of yolk carotenoids has no effect on larval development. It seems then that a major reason for mobilization of xanthophylls into the ova is to ensure that the young larvae are adequately equipped with chromatophores. If this is the major function of carotenoids, it remains to be explained why they are present in the free form in the ovaries and esterified before being transferred to the skin. In many crustacean eggs the astaxanthin is rendered water-soluble by attachment of the free pigment to a protein (Goodwin, 1951); in fish, however, no such soluble protein complexes appear to exist.

β-Carotene which is usually observed in traces in ova (Table 1) does, in the case of trout, gradually disappear during embryonic development. Steven (1949) considers that it is converted into vitamin A, and although this is entirely feasible it still requires to be proved.

In spite of the proof that carotenoids are not needed for normal embryonic development, the work of Hartmann *et al.* (1947) indicates that they may be metabolically functional and be of importance in the fertilization process rather than in embryonic development. They report that in the rainbow trout (*Salmo irideus*) astaxanthin acts as a fertilization hormone (*Befruchtungstoff*), termed gamone I (G_I). Two gamones, G_I and G_{II}, occur in trout eggs and the ovarial fluid surrounding them, G_I being thermostable and G_{II} being thermolabile and a phospholipoprotein which agglutinates spermatozoa at a dilution of $1\text{–}5 \times 10^{-9}$. Two natural antagonists (androgamones A_I and A_{II}) occur in spermatozoa; A_I occurs in the methanol extract of spermatozoa, whilst A_{II} occurs in the methanol-insoluble residue. Three types of experiment were carried out to demonstrate the G_I activity of astaxanthin. The degree and the duration of activity of spermatozoa were compared when suspended in the fluid surrounding the eggs (ovarial fluid, *Fructwasser*), in boiled ovarial fluid, and in boiled fluid to which astaxanthin, β-carotene, or lutein had been added as colloidal solutions (see Table 4). It should be noted that the inactivity of the heated ovarial fluid was due, not to destruction of G_I (which, if it were astaxanthin, would be heat stable) but to the change in pH. In this experiment, which is based on extremely subjective measurements, it must be conceded that no very striking effect is observed on addition of carotenoids in what are, in fact, relatively large concentrations. The investigators, however, concluded that this experiment demonstrated the activity of carotenoids in stimulating spermatozoal activity. The second experiment was carried out to demonstrate the chemotactic action of the pigments. In essence the method used consisted of placing two small capillaries, open at one end and filled, one with boiled ovarial fluid and the other with boiled fluid plus a colloidal solution of the pigment under investigation, in a drop of unboiled ovarial fluid to which about 1 mm.^{3} of spermatozoa was added. After about two minutes microscopic examination of the tubes was carried out. Table 5 illustrates the results obtained, astaxanthin appears to be strongly chemotactic, β-carotene slightly active and lutein inactive. In their final experiment Hartmann *et al.* (1947) examined, using the same spermatozoa-activity technique of the first experiment, the effect of these pigments in counteracting crude A_I extracted by methanol from spermatozoa. Table 6 indicates that astaxanthin, but neither lutein nor β-carotene, effectively antagonizes A_I; it is concluded then that astaxanthin is a true fertilization hormone.

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* In considering carotenoid function, a distinction must be drawn between a possible 'metabolic' function and a 'physical' function, such as participation in photo-responses, which could be carried out by the esterified pigments.

Table 4. *Degree of activity and motility times of rainbow trout spermatozoa in various media. Temperature* 10°; 1 *mm.*^{3} *spermatozoa dispersed in one drop* (0·06 *ml.*) *of medium.* [Adapted from Hartmann *et al.* (1947)]

Activity	Ovarial fluid pH 7·7	Boiled fluid pH 9·0	Astaxanthin 60 µg.	β–Carotene 120 µg.	Lutein 115 µg.
			(each of 1 ml. of follicular fluid)		
Initial activity*	3	1–2	4	3–4	2–3
Duration of free motility at max. activity (sec.)	25±3	22±2	28±1·5	28±3·5	26±1·5
Total duration of free motility	83±11	47±9	59±9·5	58±9·5	67±12
Total duration of free motility and motility without movement from one spot (sec.)	216±27	65±9	93±6·5	89±2·3	93±10·5

* Activity expressed in arbitrary units 0–4.

It must be emphasized that in this work no proof has been provided that astaxanthin is the *naturally occurring* fertilization hormone. In fact, no demonstration of its presence in the ovarial fluid which was used as the natural source of G_I was presented. All that was reported was that the fluid is pale yellow; one feels that the presence of astaxanthin in this fluid in the concentrations used in these experiments would have imparted a much stronger colour to it. A logical difficulty arises when this report is compared with earlier reports (see Kuhn & Wallenfels, 1939, 1940) on the gamones present in sea-urchin eggs. In this case echinochrome (2-ethyl 3 : 5 : 6 : 7 : 8-pentahydroxy-1 : 4-naphthaquinone) in the form of a protein complex exhibited all the properties attributed to astaxanthin in the trout. This is in spite of the fact that sea-urchin eggs are very rich in carotenoids (see Goodwin, 1951 for full details). Carotenoids were not however tested for gamone activity in sea urchins.

Table 5. *Chemotactic action of carotenoids on the spermatozoa of the rainbow trout.* (*Capillary method see p.* 76.) *Spermatozoa dispersed in ovarial fluid, pigments dispersed in boiled ovarial fluid.* (Adapted from Hartmann *et al.*, 1947) *

Contents of capillaries		Observation
Capillary I	Capillary II	
Boiled ovarial fluid	Boiled ovarial fluid	Equally few spermatozoa in each capillary
Astaxanthin (60 μg./ml.)	Boiled ovarial fluid	About five times as many spermatozoa in I than in II. Accumulation at the closed end of I. Accumulation and increased activity at open end of I
β-Carotene (120 μg./ml.)	Boiled ovarial fluid	About three times as many spermatozoa in I than in II, otherwise activation effect as with astaxanthin
Lutein (115 μg./ml.)	Boiled ovarial fluid	No differences between I and II
Astaxanthin (60 μg./ml.)	β-Carotene (120 μg./ml.)	About 1·5 times as many spermatozoa in I; activation and accumulation at open ends of capillary in both cases
Astaxanthin (60 μg./ml.)	Lutein (115 μg./ml.)	About five times as many spermatozoa in I

The whole problem of the function of carotenoids in reproduction is at the moment obviously in the melting-pot, and further developments should considerably clarify the position, although it would be hazardous to foretell the final outcome. The mass of heterogeneous information at present available on this topic has recently been reviewed by Goodwin (1950 *a*).

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* Lord Rothschild (1951) has informed the writer that he has not been able to confirm the observations of Hartmann *et al.* (1947) and that he considers their work open to severe criticism.

Table 6. *Connection between gynogamone I (astaxanthin) and androgamone I. 5 mg. of crude androgamone I in 1 ml. water, pigment dispersed in boiled ovarial fluid.* t *= total duration of free motility.* t_0 *= total duration of free mobility and motility without movement.* (Adapted from Hartmann *et al.*, 1947)

Medium	Ratio of medium to crude androgamone	Observations on spermatozoa			Remarks
		Activity	t	t_0	
H_2O	1 : 0	1	34	47	
	3 : 1	—	—	—	Completely inactive
	9 : 1	1	25	45	
Boiled ovarial fluid	1 : 0	2	30	70	
	3 : 1	1	28	95	
»	1·5 : 1	—	—	—	Occasional free movement
	1 : 1	—	—	—	Completely inactive
	1 : 12·5	—	—	—	
Astaxanthin (60 μg./ml.)	1 : 0	4	28∓15	93∓6·5	
	3 : 1	3	25	100	
	1·5 : 1	3	34∓6	104∓15	
	1 : 1	3	25	92	
	1 : 12·5	2	—	20	30% of spermatozoa active
	1 : 50	—	—	—	Completely inactive
β-Carotene (120 μg./ml.)	1 : 0	3	28	89	
	3 : 1	2	32	60	
	15 : 1	—	—	—	Occasional activity without translocation
	1 : 1	—	—	—	Completely inactive
Lutein (115 μg./ml.)	1 : 0	2	26	93	
	5 : 1	—	—	—	Completely inactive
	1 : 1	—	—	—	

THE SOURCE OF VITAMIN A IN FISH

It is appropriate to consider this problem here not only because it is intimately concerned with carotenoid metabolism in fish but also because recent investigations have brought the problem much nearer solution.

Small fish, in the main, live on zooplankton, the permanent source being mostly copepods, whilst the 'transitory' source consists mainly of eggs and larvae of both invertebrates and fish ; phytoplankton are also utilized to some extent (Johnson, Scott & Chadwick, 1934). The major pigment of copepods, which live on phytoplankton, mainly diatoms, is generally accepted as astaxanthin (Euler, Hellström & Klussmann, 1934 ; Lederer, 1935 ; Sörensen, 1937 ; Goodwin & Srisukh, 1948 ; Kon & Thompson, 1949 *a*, *b*), only small amounts of β-carotene being occasionally encountered. The claim of Wagner (1939) to have obtained crystalline β-carotene from the 'krill' (mixed copepods) of northern whales is probably incorrect, although, as pointed out by Moore (1950), the variations in the relative amounts of β-carotene and astaxanthin observed at different stages of development of locusts (Goodwin, 1949) may possibly apply to copepods. The copepods probably manufacture astaxanthin by oxidation of the diatom carotenoids.

It has long been realized that the very small amounts of β-carotene in zooplankton were insufficient to account for the large sources of vitamin A which zooplankton-eating fish accumulate not only in the liver but in the intestinal wall. It has been suggested that astaxanthin might be a vitamin A precursor in fish (Morton, 1940) but on general grounds this must be considered highly improbable (see Goodwin, 1950 *b*) ; it should be emphasized, however, that the suggestion has not yet been experimentally disproved. In fact, very recently it has been claimed that astaxanthin isolated from the shrimp, *Aristeomorpha foliacea* (= *Penaeus foliaceus*), is vitamin A-active ; on conversion into astacin this activity is lost (Grangaud & Massonet, 1950). This work deserves repetition because no account was taken of the possible presence of vitamin A in the astaxanthin fraction and the method of producing astacin would remove any vitamin A present. Assuming that astaxanthin is inactive, two further possibilities remain, either (*a*) the diet (zooplankton) contains pre-formed vitamin A or (*b*) fish synthesize vitamin A *de novo*. The latter suggestion has always appeared improbable, for no other animal species can carry out this synthesis, but it is only very recently that the first alternative has been unequivocally proved.

Pioneer investigations in this sphere were carried out by Drummond & Gunther (1934), who found that the oils obtained from mixed copepods and *Calanus finmarchus* showed very little vitamin A activity when tested biologically ; it seemed that this slight effect was due to traces of β-carotene, for no chemical demonstration of the presence of vitamin A could be made. Phytoplankton from *Chaetoceros* spp. and *Lauderia borealis*, on the other hand, were more active owing to their higher β-carotene content.

In 1939, Gillam, El Ridi and Wimpenny carried out an exhaustive study of gross plankton hauls from the North Sea. Both β-carotene and vitamin A were detected and, as the main components of the phytoplankton fraction, *Rhizosolenia styliformis* and *Bidulphia sinensis*, contained no vitamin A, it was not unreasonable to assume that ' by difference ' the vitamin A came from the zooplankton. However, on examining plankton hauls at intervals during the year, it was found that the vitamin A content was maximal well before the zooplankton population reached its peak. Because of this, Gillam *et al.* (1939) quite rightly refused to consider the presence of vitamin A in copepods as proved, and it was not until ten years later that, largely owing to the considerable improvements which had taken place during this period in chromatographic and spectrographic techniques, the problem was finally solved. Nielands (1949) found considerable amounts of vitamin A in the hepatopancreas and eyes of the common lobster (*Homarus vulgaris*) and Kon & Thompson (1949 *a*, *b*) at almost the same time showed that it was present in a number of species of smaller crustacea (typical zooplankton), being apparently concentrated in the exoskeleton. Nieland's work also showed that the lobster possessed the ability to convert β-carotene into vitamin A, although under his experimental conditions the conversion was not particularly efficient.

Recently, Lane (1950) has also separated the vitamin A activity of zooplankton (*Temora turbinata* and *Centropages typians*) lipids from the carotenoid fraction. The vitamin A-active fraction, which had an absorption spectrum with a maximum at 310 mμ., when fed to the fish *Limanda ferruginea* resulted in the accumulation of vitamin A ($\lambda_{\text{max.}}$ 325 mμ.) in the liver. Lane therefore concludes that a non-carotenoid material can be utilized in fish as a vitamin A precursor. It is felt that more evidence of the purity of Lane's fraction must be provided before final assent can be given to this conclusion. From the details so far published, one is justified in assuming that the 310 mμ. maximum is due to vitamin A, but that the presence of considerable amounts of impurities has caused a shift in the usual position of the maximum.

There is yet no evidence that freshwater zooplankton contains pre-formed vitamin A, although it is extremely likely. In this connection it will be interesting to see if freshwater copepods contain considerable amounts of vitamin A_2, a characteristic of freshwater fish. Although there now appears to be no doubt that fish obtain most of the vitamin A pre-formed, it must not be overlooked that they also have the ability to convert β-carotene into vitamin A and vitamin A_2. Formation of vitamin A was recently demonstrated by Nielands (1949) in the case of the Atlantic cod (*Gadus callarias*), whilst the formation of vitamin A_2 in dace and perch was observed in 1939 by Morton and Creed.

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6. BILE SALTS OF FISH

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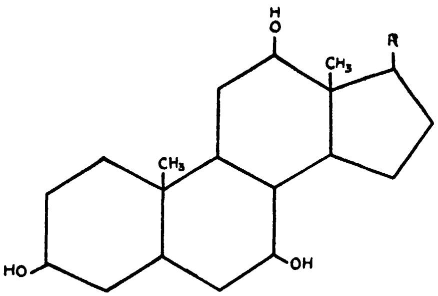
I. DISTRIBUTION

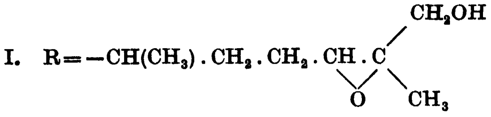
(a) *Elasmobranchii.* The distribution of different bile salts in this subclass is shown in Table 1.

Table 1. *Bile salts of elasmobranch fish*

Animal	Compounds in bile	Conjugate	Reference
Class PISCES			
Subclass ELASMOBRANCHII			
Carcharinidae. Tiger shark, *Galeocerdo arcticus*	Scymnol	Sulphate	Bergmann & Pace (1943)
Squalidae. Spiny dogfish, *Squalus acanthias*	,,	,,	Cook (1941)
Scymnidae. Shark, *Scymnus borealis*	,,	,,	Hammarsten (1898)
Isuridae. *Isuropsis glauca*	,,	,,	Oikawa (1925)
? Two species of shark	Scymnol; cholic and a C_{27}-acid		Ohta (1939)
Rajidae. Skate (blue), *Raia batis*	Scymnol	,,	Cook (1941)
Trygonidae. 'Akajei' fish, *Dasyatis akajei*	Scymnol, cholic acid	,,	Ashikari (1939)

Scymnol has been isolated from the bile of all species examined. This substance has been assigned the formula I (steric relationships not indicated), which is that of tetrahydric alcohol containing an oxide ring and having the entire hydrogenated carbon skeleton of cholic acid (II): in fact scymnol has been converted to cholic acid. The main natural bile salt of the Elasmobranchii examined is apparently a sulphuric acid ester or mixture of such esters from which, by alkaline hydrolysis, scymnol is derived. It has never been satisfactorily proved that scymnol itself occurs (conjugated) in the bile, for it is conceivable

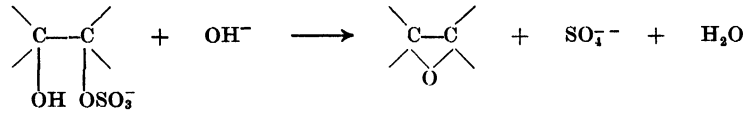



I. $\mathrm{R}=-\mathrm{CH(CH_3).CH_2.CH_2.\underbrace{CH.C}_{O}(CH_2OH)(CH_3)}$

II. $\mathrm{R}=-\mathrm{CH(CH_3).CH_2.CH_2.COOH}$

that the oxide ring might arise during hydrolysis of a sulphuric ester of a glycol or other diol :

e.g.


$$\underset{\mathrm{OH}}{\mathrm{C}}-\underset{\mathrm{OSO_3^-}}{\mathrm{C}} + \mathrm{OH^-} \longrightarrow \underbrace{\mathrm{C}-\mathrm{C}}_{\mathrm{O}} + \mathrm{SO_4^{--}} + \mathrm{H_2O}$$

Scymnol is a substance closely allied to cholic acid, and contains also the C_{27} carbon skeleton of cholesterol.

The claims to have detected cholic acid in the bile of sharks and of the ray, *Dasyatis akajei* (see Table 1), are of great interest and ought to be re-examined. The occurrence of a C_{27}-acid in elasmobranch bile is perhaps not so surprising (see Discussion).

(b) *Teleostei.* A table showing the distribution of different bile salts in teleostean fish has been compiled by Haslewood & Wootton (1950) and indicates that the bile cf about 39 species has been examined. In all cases where acids were identified, cholic acid was claimed to be present, whilst chenodeoxycholic acid was reported in 17 species. In four cases a tetrahydroxy acid was said to occur. Most of this work was carried out in Japan, with species found in Japanese waters. The present speaker has confirmed the presence of cholic acid as almost, if not entirely, the sole bile acid in the plaice and cod, but has found that in the Mirror carp (*Cyprinus carpio*, var.) the bile salts consisted largely of neutral material, conjugated with sulphate, as in the Elasmobranchii. This is the more surprising, as the Japanese workers have claimed that the bile of *Cyprinus carpio*, as well as that of the closely related goldfish, *Carassius auratus*, contains cholic acid, which I have not so far been able so isolate from Mirror carp bile : if it is indeed present there, it can constitute only a small proportion of the bile salts.

II. DISCUSSION

The reported work clearly indicates that the characteristic bile salt of Elasmobranchii is probably scymnol sulphate or some closely related substance. That is to say, it seems that these fishes are deficient in the power to effect the synthesis of a C_{24}-bile acid. A similar state of affairs exists in the frogs and toads examined, whose bile salts appear mainly to follow a similar pattern (C_{27}-alcohols, conjugated with sulphate) to those of the Elasmobranchii. It has already been suggested (e.g. by Haslewood & Wootton, 1950) that such bile salts, as well as those containing C_{27}-acids, may indicate that the species possessing them are evolutionarily of a primitive type. Cook (1941) has referred to a suggestion of Windaus who, assuming that both scymnol and the C_{24}-bile acids may be derived ultimately from cholesterol, put forward the view that the Elasmobranchii may lack the power of oxidation of the sterol side-chain. It is partly this suggestion that makes it important that the claim for the existence of cholic acid also in Elasmobranch bile should be carefully re-examined.

If it is accepted that the possession of cholic acid or one of its near C_{24}-relations is a ‘ modern ’ characteristic, then clearly many, if not most, of the Teleosteii are modern in that sense. It will, however, be of great interest if it can be shown that the bile salts of the carp and possibly other teleostean species are of the C_{27}-alcohol-sulphate type or contain C_{27}-acids.

It has also been pointed out that certain species or groups of closely related species have unique bile-acids : examples are phocaecholic acid from the bile of Pinnepedia (seals and walruses), pythocholic acid from the Boidae (boas and pythons), hyodeoxycholic acid from pigs' bile and nutriacholic acid from *Myocastor coypu* (nutria rat). The reported occurrence, therefore, of tetrahydroxy acids in some teleostean biles may be a further indication that such uniqueness will be found in this group also, and a chemical examination of such unusual acids may show that a knowledge of their distribution is of value in the classification of fishes.

It would be desirable to confirm the existence of chenodeoxycholic acid in teleostean bile. On quite inadequate evidence (only six species having been examined) it is often stated that this acid is characteristic of the bile of birds, although it is also found in certain mammals, including man.

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7. ECONOMIC ASPECTS OF THE UTILIZATION OF FISH

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WORLD PRODUCTION

World fishery statistics are inadequate and incomplete, but an estimate of pre-war production was made a few years ago by the U.S. Fish and Wildlife Service (Sandberg, 1944), and the Food and Agriculture Organization of the United Nations is now systematically collecting and publishing up-to-date figures (Food and Agriculture Organization of the United Nations, 1948 *b*, 1949 *a*, *b*, *c*, 1950). Some of these are summarized in Table 1 and compared with pre-war estimates. The estimated pre-war catch was between 17 and 18 million tons and, after a relapse during the war, current estimates are again of the same order. Much subsistence fishing throughout the world must have been overlooked or unaccounted for by official statistics, which often record only exportable surpluses, and 20 million tons has been taken as an accurate enough round figure for total production.

It will be seen that Japan is the world's largest fish producer. Before the war it produced 22% of the estimated world catch, and China and Korea, also fishing East Asian waters, accounted for about 13%; U.S.A., including Alaska, with 11%, U.S.S.R. with 9·3%, and U.K. with 6·4%, also landing more than one million tons each. Since the war, Norway has moved up to fourth place with 8·7% of the estimated 1948 world catch.

The estimated value of these catches is not proportional to the total landings, because some fish are worth more than others and the price obtained also depends on the organization of marketing.

The numbers of fishermen employed also vary enormously, depending on the organization and efficiency of the industry. Before the war, Japanese fishermen numbered over a million, so that average output was only about 3 tons per man-year, whilst Iceland only employed about 5,000 with an average output of nearly 100 tons per man-year. The latter is also about the average output from North Atlantic trawling, whilst the Californian pilchard fishing, at its peak, yielded nearly 250 tons per man-year. Generally speaking, fish can be produced at a lower cost than protein foods on land.

Table 2 analyses the pre-war catch by oceans, hemispheres and continents. Although about 70% of the earth's surface is sea, fishing-grounds, workable by the normal variations of line and net, only occur in relatively shallow waters, down to at most 300 fathoms in the case of the largest trawlers, and this restricts the area of operations of commercial fisheries. Some 93% of the world's fish was caught north of the Tropic of Cancer, mostly in the North Atlantic and Pacific Oceans as far North as latitude 75°, which is well inside the Arctic Circle. Asia alone accounted for about one-half of the total catch. It appears, however, that there may be suitable fishing-grounds

Table 1. *World production of fish*

Country	Present Population (millions)	Typical Pre-war Catch (thousands of metric tons)	Estimated Value (millions of U.S. dollars)	Year	Post-war Catch Quantity (thousands of metric tons)
Japan	80	3,678	101	1949	2,923
U.S.A. and Alaska	148	1,844	99	1949	2,523
U.S.S.R.	212	1,557	82	1948	1,555
China	450	1,311*	20	—	—
U.K.	50	1,060	81	1949	1,152
Korea	50	1,048	24	1949	295†
Norway	3	926	24	1949	1,166
India, Iran, Burma	430	907	20	1946	660‡
Germany	66	724	42	1949	471
Canada	13	544	30	1949	605
Indonesia	76	472	—	—	—
Spain	28	439	68	1948	523
France	41	357	46	1949	384
Iceland	0·1	336	16	1949	394
Indochina	27	266	—	—	—
Newfoundland	0·3	204	8	1949	266
Netherlands	10	159	—	1949	236
Italy	46	138	—	1949	152
Sweden	7	133	—	1949	201
Portugal	8	118	—	1949	283
Denmark	4	114	—	1949	255
Belgium	9	—	—	1949	68
Finland	4	—	—	1949	66
British Malaya	5	89	—	—	—
Siam	18	—	—	1949	196
Philippines	19	81	—	1949	76
Mexico	24	70	—	1949	55
Chile	4	—	—	1949	76
Brazil	48	61	—	—	—
Kwantung	1·2	60	—	—	—
Argentina	10	55	—	—	—
Venezuela	4	45	—	—	—
All others	—	633	—	—	—
TOTAL		17,690	—	—	—

* Another estimate 2,700.
† South Korea.
‡ India only.

on the continental shelves in the Southern Hemisphere, off South America and South Africa, which are not yet being fully exploited. Fish populations seem less dense in the warmer tropical and sub-tropical waters. Although there appear to be many more species and there may even be more marketable species, the total marketable catch is smaller.

Table 2. *Analysis of pre-war world fishery production*

Water	% of total	Hemisphere	% of total	Continent	% of total
North Atlantic	46	Northern	98	Asia	49
North Pacific	47	Southern	2	Europe	32
Indian Ocean	5			North America	16
South Atlantic	1			Rest	3
South Pacific	1				

A recent estimate (Thompson, 1949) of the possible increase in the catch of world fisheries is that about 2·7 million tons extra might be obtained from cold and temperate waters, and perhaps 1·3 millions from tropical and sub-tropical waters, making a 22% increase in the pre-war catch. A more optimistic estimate (Lucas, in the press) puts the forseeable increase at 5 to 10 million tons.

WORLD CONSUMPTION

By no means all of the world's fish catch goes into direct human consumption. A fair proportion, perhaps about one-third, chiefly of the fatty fishes, is used for agricultural or industrial purposes, leaving some 13 million tons for human food. A proportion of this consists of heads and bones, which are normally inedible. Fish is presented to the consumer in a variety of forms, e.g. headed, steaked, filleted, etc., all of which result in varying degrees of wastage. Filleting of white fish at the port is very common in Britain and U.S.A. If hand filleted, the losses vary mostly from 40 to 60% according to species (Reay, Cutting & Shewan, 1943). There is also a seasonal variation for any one species, depending on the maturity of the sexual organs and the plumpness of the flesh itself. In addition, the yield from fish of the same species at any one time is very variable and depends on the skill of the operative, etc. When centralized at the port, the filleters' offal is converted into white fish-meal for animal feeding.

Flesh can be removed more cleanly from cooked fish than from raw fish. White fish offal contains quite a high proportion, perhaps about one-third, of adhering flesh. Attempts have been made to recover this for human consumption (Lantz, 1947, 1948). The percentage of wastage would be different again in the case of processed, e.g. salted or smoked fish, whilst the bones of canned fish can, of course, be eaten without difficulty.

It would be accurate enough for most purposes to assume an average figure of 50% for the inedible portion, which results in a figure of about 6·5 million tons of fish flesh consumed annually.

The average protein content (i.e. protein nitrogen × 6·25) of most fishes varies from about 14 to 16·5%. Total nitrogen × 6·25 gives a figure of 16 to 20% owing to the relatively high non-protein nitrogen content of fish flesh. Taking 15% as an average, the world annual consumption of fish protein is about one million tons, corresponding to an average of about 500 g. per head per annum, which is only about 2% of the estimated total annual world consumption of 50 million tons of first-class protein.

Fish is principally a source of 'first-class' protein with additional but fluctuating fat content in some classes such as herrings (see Lovern & Wood, 1937) and their allies, which consequently have a higher energy value. Apart from the livers of certain species, which are not eaten as such, vitamin potency and mineral content are relatively of secondary importance.

Lovern (1944) has estimated on various assumptions that in 1938 the average annual *per capita* contributions of fish to the British diet were:—protein, 3·2 lb. (1,450 g.), of which 2·4 lb. was from the demersal, 'white' fishery and 0·8 lb. from herring; fat, 1·7 lb. (770 g.), of which 0·8 lb. was from herring and 0·9 lb. from white fish livers. The daily consumption of vitamin A from liver oils of cod, etc. was 1,154 I.U. and from halibut liver oil 84 I.U.; and of vitamin D, 261 I.U. from herring body and cod-liver oils and 3·6 I.U. from halibut livers. By comparison, the Antarctic whale fishery in 1938 yielded 4·9 lb. (2,220 g.) of fat per annum and 624 I.U. vitamin A per day.

It has further been calculated (Reay *et al.*, 1943) that British fish could thus only have accounted for about 10% of the animal and 5% of the total protein in our pre-war diet and about 2% of the total fat consumed, which together contributed between 30 and 40 calories per day.

The consumption of fish flesh per head of the population varies tremendously from one area of the world to another, from about 1 kg. (2¼ lb.) of edible flesh per year in the countries of Central and Eastern Europe to as high as 45 kg. (about 100 lb.) in the case of the Philippines and Ryukyu Islands of Eastern Asia. F.A.O. has sifted and summarized existing data (Food and Agriculture Organization of the United Nations, 1949 *a*) and placed countries into four categories, as shown in Table 3, which includes a typical estimate for the consumption of each country.

The distribution inside countries such as India and China, which have low average consumptions, must be very uneven, some classes of people living along the sea coast or rivers having a relatively high intake and inland dwellers getting practically none at all. Even in the West Indies, where one might have expected a uniformly high level of consumption, there appear to be large variations amongst the islands.

Table 3. *World consumption of fish* (*approximate*)

(kg. per head of edible flesh per annum)

1. *Very high* (20 kg. or more)	Philippines	45
	Ryukyu Islands	45
	Burma	40
	Japan	30
	Iceland	30
	Norway	25
	Sweden	25
High (10–19·9)	Belgium	10
	Denmark	18
	Germany	7
	Netherlands	10
	Portugal	13
	Spain	8
	U.K.	15
	Korea	18
	Siam	10
	Indochina	12
	West Indies	15–40
	Malaya	20
3. *Intermediate* (5–9·9 kg.)	Finland	7
	France	5·5
	Greece	7
	Italy	5·5
	Luxembourg	5
	Australia	5
	Ceylon	5
	New Zealand	6
	Canada	5
	Mexico	5
	U.S.A.	5
	Chile	11
	Venezuela	10
	Gold Coast	9
4. *Very low* (< 5 kg.)	Eastern Europe	
	Ireland	
	India and Pakistan	
	China	
	Java	
	Turkey	
	South and Central America	
	Bahamas	
	Cuba	
	Dominica and Haiti	
	North, East and South Africa	

In some large fish-producing countries, such as Norway and Iceland, fish consumption is high, 25–30 kg. of edible flesh per year. In others, such as Canada, it is relatively low (5–10 kg.) and there is, taking 1947 figures, a much higher consumption of meat (66 kg. per year), poultry (9·7) and eggs (14·9). The average U.S. consumption has remained remarkably steady at between 4 and 5 kg. per year since 1930. In the U.K. the estimated consumption has risen from 12·1 for

1934–1938 to 15·1 for 1947–1948. Whilst the total amount of fish now available is second only to meat as a first-class protein food, the ratio of the gross weights of fish to meat has been increased by one-half (Central Statistical Office, 1948).

In 1946 the gross home production of fish was somewhat greater than that of meat and a good deal greater than that of all other protein foods put together.

THE PRINCIPAL WORLD FISHERIES

There must be many thousands of marketable commercial species. In Britain alone there are more than 50, the catch of the principal ones being given in Table 4.

Nevertheless, about one-third of the world's catch can be accounted for by about six major species. Table 5 summarizes the data on the distribution of the world catch of various species of fish. There are inconsistencies in the available statistics and difficulties and may be errors in interpretation, but the general picture presented is substantially correct. There are two main types: pelagic fish, which are often caught by gill nets, etc. when shoaling near the surface of the sea, and are usually fatty, e.g. herring and pilchards; and demersal fish, such as cod, plaice, etc. (' white ' fish), which are usually caught on the sea bottom by trawl-nets or baited lines.

The most prolific species of all is the herring, of which over 1·8 million tons were landed annually in Northern and Western Europe alone in the pre-war years. Including catches off the Western Atlantic coast (where it accounted for 38% of the total Canadian catch), and of similar species in the Pacific, the world catch of herring is now about 15% of the total (Food and Agricultural Organization of the United Nations, 1948 *b*). The largest single catches of any species by any one country are the Norwegian herring catch which has exceeded one million tons, and the Japanese catch of pilchards which, although variable, has exceeded 1½ million tons. The Californian pilchard fishing which before the war amounted to 25% of the total U.S. catch is also rather erratic and seems to be in decline at present, partly perhaps owing to the effects of 'over-fishing'. Before 1914, the U.K. caught over 500,000 tons of herring, representing rather more than half the total catch (see Table 6), but largely owing to export trade difficulties the industry declined between the wars, and is now less than 250,000 tons and one-fifth of the catch.

The menhaden, too, is important, although confined to the east coast and gulf states of U.S.A., where it constituted 18% of the pre-war catch and is now approaching 500,000 tons.

Most of the pelagic fish catch is used for animal feeding and industrial purposes. More important as a direct contribution to the human diet and pre-eminent amongst the demersal fisheries is the catch of cod and related 'gadoid' (i.e. 'codlike') species of the North Atlantic and to a much lesser extent, in the North Pacific.

Table 4. *Approximate quantities of British fish landed in* 1949

(thousands of tons)

Kind	England	Scotland	Total
DEMERSAL			
Gadoid (cod-like) fish :			
Cod	340	42	382
Haddock	84	47	131
Whiting	15	21	36
Saithe (coalfish)	26	6	32
Ling	7	5	12
Tusk	—	3	3
Hake	23	5	28
			— 624
Flat fish :			
Plaice	31	5	36
Halibut	2	4	6
Dabs	4	1	5
Turbot	3	1	4
Lemon soles	1	3	3
Dover soles	3	—	3
Megrims	3	1	4
Witches	1	1	2
			— 63
Cartilaginous fish :			
Skate and rays	14	5	20
Dogfish	11	—	11
			— 31
Miscellaneous :			
Redfish	12	—	12
Catfish	7	—	7
Gurnards	4	—	4
Monks (anglers)	1	2	3
Conger eels	2	1	3
Other kinds, unclassified and mixed	3	3	6
Livers and liver oil	26	1	27
Roes	2	1	2
			— 64
Total demersal	628	158	786
PELAGIC			
Herring	72	130	201
Mackerel	3	4	7
Sprats	2	2	4
Pilchards	3	—	3
Total pelagic	80	135	215
Total wet fish	709	293	1002

Owing to figures being rounded off to the nearest 1,000 tons, the totals do not always agree with the sums of the items.

The cod is the principal species of commerce and F.A.O. has recently published a report which collates much interesting statistical data (Gerhardsen & Gertenbach, 1949) (see Tables 7 and 10).

Table 5. *Some of the principal fisheries of the world (thousands of tons for a typical pre-war year and 1948, unless specified)*

Country	Herring			Pilchard		Menhaden		Tuna		Salmon		Flat fishes		Elasmo-branchs		Freshwater species		Other fish		Shellfish	
	1920	1938	Post war	Pre war	Post war	Pre war	Post war	Pre war	Post war	Pre war	Post war	Pre war	Post war	Pre war	Post war	Pre war	Post war	Pre war	Post war	Pre war	Post war
Denmark	—	16	52	—	—	—	—	—	—	—	—	30	61‡	—	—	—	—	—	—	—	—
France	—	72	50‡	—	—	—	—	—	—	—	—	11	—	—	—	—	—	—	—	—	**43**
Germany	60	266	173	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Iceland	20	130	150	—	—	—	—	—	—	—	—	2	8	—	—	—	—	—	—	—	—
Netherlands	—	91	150	—	—	—	—	—	—	—	—	14	25	—	—	—	7	—	—	**66**	**63**
Norway	280	600	1033	—	—	—	—	—	—	—	—	8	7	11	25	—	—	—	—	**7**	**10**
U.K.	550*	285	264	—	—	—	—	—	—	—	—	64	108‡ 74	33	33	—	—	**38**	**42**	**31**	**27**
Canada																					
Atlantic	—	150	175	—	—	—	—	—	—	—	—	11	20	—	—	39	36	—	—	**33**	**36**
Pacific	—	35	58	47	—	—	—	—	—	67	65	—	—	—	—	—	—	—	—	—	—
Newfoundland	—	12	42	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
U.S.A.																					
Atlantic	14–40		86	—	—	270	485	—	—	—	—	57	73‡	6	17	62	76‡	**148**	**269‡**	**614**	**596**
Pacific	20–100		79	500	163	—	—	83	119‡	300	226‡	—	—	—	—	—	—	—	—	—	—
Mexico	—	—	—	—	—	—	—	—	54†	—	—	—	—	—	—	—	—	—	—	—	—
Japan	40–1000		229‡	400- 1650	559	—	—	203	86‡	221	14‡	63	112‡	65	75‡	11	30‡	**619**	**542‡**	**419**	**750**

* 1913.
† 1946.
‡ 1947.
§ 1949.
|| For cod, etc. see Table 7.

The U.K. which has the largest trawling fleet in the world, accounts for about half of the total for North-west Europe, with nearly 750,000 tons yearly, about half of which is cod. The Grand Banks, off Newfoundland, exploited largely by means of lines, yielded before the war about 450,000 tons. Most of the North Atlantic countries shared in the cod fishery, which has spread to more distant waters since the 1920's. Cod itself probably accounts for about two million out of 2·5–3 million tons of total gadoids. Britain easily tops the list for gadoids other than cod, such as haddock, which is Scotland's most important demersal species, caught mainly in the near and middle waters.

Table 6. *Yield of British marine fisheries*
(thousands of tons)

	1913	1938	1947	1949
Herrings, etc.	588	269	220	215
White fish	555	757	764	786
Total	1,143	1,026	984	1,002

Next in importance come various species of salmon and tuna (Food and Agricultural Organization of the United Nations, 1949 *c*), amounting to about 300,000 tons each annually, and important as exports, chiefly in the canned form.

The fishes to which we normally give pride of place are flat fishes, of which there are numerous species, plaice being the most numerous one taken off North Western Europe. The U.K., Japan, U.S.A. and, since the war, Denmark are the greatest producers, accounting for about a quarter of a million tons out of a total of some 400,000 tons. The U.S. 'rose-fish' industry has developed in recent years to more than 100,000 tons as a result of the extensive marketing of fillets. Further vast unexploited reserves of this fish may exist, but these will be technically difficult to exploit (Beverton, 1947). The elasmobranch species, skates, dogfish, sharks, etc., account for perhaps 150,000 tons, of which Japan contributes about half.

Freshwater species, important for inland countries such as Czechoslovakia and countries with many lakes, such as U.S.A., Canada and Finland, as well as ones where aquiculture is practised, e.g. Japan, account for perhaps a further 250,000 tons. Aquiculture may be expected to increase in tropical countries. Shellfish contribute considerably to the gross weight, mostly shell of course, of fish produced by U.S.A. (0·5 million tons) and Japan (0·75 million tons) out of a total of perhaps 1·5 million.

Japan is credited with large quantities of miscellaneous fish not included under the above headings (0·5 million) and miscellaneous marine products such as sea-weed and sea-urchins, etc. (0·5 million tons).

In this way, about two-thirds of the world's production of fish can be accounted for.

Table 7. *Landings of cod and related species from principal Atlantic fishing-grounds*

Country	Total landings						North Sea				Norwegian Sea				Spitzbergen and Bear Island				Barents Sea				Iceland				Greenla	
	1920		1938		1948		1920		1938		1920		1938		1920		1938		1920		1938		1920		1938		1920	1
	Cod	Others	Cod	Others	Cod	Others	Cod	Others	Cod	Others	Cod	Others	Cod	Others	Cod	Others	Cod	Others	Cod	Others	Cod	Others	Cod	Others	Cod	Others	Cod	C
England	127		327	89			52	157	21	19	0	0	10	7	0	0	123	9	0	0	44	26	56	34	107	28	—	–
Scotland	48		28	48			31	60	19	46													8	3	1	2		
Total U.K.	175		355	379	338	396	83	217	40	65	0	0	10	7	0	0	123	9	0	0	44	26	64	37	108	30		
Denmark	17		19	10	55†		2	10	3	1																		
Faroe Is.	27		58												0	0	7	0					19	0	22	1	0	[illegible]
France	98		175	19	80														0	0	1	0	22	0	15	0	82	1
Germany	32		179				9	43	3	12	0	0	33	32	0	0	27	5	0	0	28	20	19	18	35	46		
Iceland	83		132		145	136																						
Netherlands	4		6	13	13																							
Norway	186		344	92	134	221	30	7	5	12	98	22	266	57	39	10	—	—	0	0	60	28	0	0	5	0	*	
Poland					31																							
Portugal	11		40		68																						11	4
Spain	—		19		68†																						0	1
Sweden	6		13	19		45																						
Canada	110		95	28	113	63																					110	9
Newfoundland	243		233		308																						243	2
U.S.A.	219		59	130	41	31																					49	5
Japan			194		188																							
Grand total	1046		1743	1360	2032		129	244	58	110	98	22	309	97	39	10	158	14	0	0	135	73	208	73	320	92	496	63
Total Atlantic	970	—	1614	—																								
Total N.E. Atlantic	—	348	(1120)	385																								
Quantitysalted	833	17	834	12																								
% of total salted	85·9	4·9	51·7	3·1																								

* 1946. † 1947.

PRESERVATION, MARKETING AND DISTRIBUTION

Once caught, the fish usually has to be preserved by some means, so that bacterial decomposition is slowed down or arrested, whilst it is being distributed prior to ultimate consumption.

Table 8, taken chiefly from various F.A.O. estimates (Food and Agricultural Organization of the United Nations, 1948, 1949 *b*, 1950), shows the recent general picture for the distribution of fish in certain fish-producing countries, accounting for about one-half of the world's catch. Estimated allocations are given alongside the weight of product, which is usually smaller owing to trimming, wastage, etc., and once again inconsistencies appear.

Primitive and traditional processes, such as salting and drying, which require practically no capital expenditure, are still carried on in a large way with the two most important species of the western world, cod and herring. These processes, although their product is perhaps losing favour in the more industrialized nations, may, suitably modified, still prove capable of expansion and development for some time to come, as a means of feeding the under-nourished populations of tropical and undeveloped countries.

Herrings, salted and packed in barrels in the brine produced, as developed by the Dutch in the 14th century, keep for several months and are still relished in Central Europe. Britain used to be the principal suppliers before 1914, when 300,000 out of its catch of 550,000 tons was treated in this way. This export declined between the wars (see Table 9), largely owing to foreign competition and trade barriers, and partly perhaps because of the development of more discriminating palates that no longer relish the bitter, salty and ' cheesy ', ' cured ' flavour, since a greater variety of milder flavoured commodities has been made available by the processes and communications of the Industrial Era. There has been a further steep decline in the output of salt herrings since the war.

' Red ' herrings, also a medieval product, which are smoked until hard after salting, now have only a limited market chiefly in Mediterranean countries, although this might well be expanded. Kippered and bloatered herrings and the equally important smoked white fish (accounting for about one-eighth of the total white fish landings) (Reay, 1949), which play a relatively large part in the British diet, as well as the hot-smoked, cooked products of continental Europe and America, are more sophicated products, in which the smoke is more of a flavouring than a means of preservation. They require an organized rail or road transport network for quick distribution.

Cod is still dried without salt in the sun and wind in Norway to produce ' stockfish ', of which 20,000–30,000 tons used to be exported annually to West Africa. At the peak of this trade in 1926, the allocation was as great as that for dried salted cod, namely about 135,000

Table 8. *Principal cutlets for fish*

(thousand metric tons)

Country	Year	Fresh and frozen					Dried, salted and smoked					Canned				Oil and meal						Miscellaneous	
		Freshing		Freezing		Total wt. of fresh and frozen products	Total allocation	Production				Allocation	Production			Cod-liver oil	Allocated to fish-meal and oil	Herring		Other fish oil	Other fish-meal	Misc. uses	Waste
		Allocation	Product	Allocation	Product			Dried fish	Salted cod, etc.	Salt herring, etc.	Smoked herring, etc.		Salmon	Herring, etc.	Tuna, mackerel, etc.			Meal	Oil				
Canada	1948	137	104	92	50	158	129	—	23	11	—	102	29	20	2	1	152	31	12	—	10	—	—
Denmark	1947	—	90	—	3	109	—	—	2	7	4	—	—	2	2	—	—	—	—	11	5	—	—
France	1948	287	257	29	—	—	113	—	28	30	6	34	—	13	11	1	—	—	—	—	—	—	—
Iceland	1948	174	134	94	32	166	44	—	11	11	—	1	—	—	—	9	149	23	17	5	5	4	—
India	1946	330	—	—	—	—	262	125	137‡	—	—	—	—	—	—	—	68	—	—	—	—	—	—
Italy	1947	—	112	—	—	112	—	—	—	10	—	—	—	6	7	—	—	—	—	—	—	—	—
Japan	1947	1453	—	—	50	—	—	7	7	70	—	—	—	1	—	—	—	3	2	—	1	—	—
Netherlands	1948	122	71	3	1	72	126	—	—	74	25	14	—	5	—	—	4	—	—	—	6	1	—
Newfoundland	1948	15	8	36	12	21	318	—	48	20	—	1	—	—	—	2	5	1	1	—	—	—	—
Norway	1948	237	227	42	42	273	318	10	30	110	—	58	—	41	2	7	744	110	40	8	14	22*	31
Portugal	1947	—	—	—	—	97	—	—	23	3	—	—	—	38	—	—	—	—	—	—	—	—	—
Spain	1949	—	—	—	—	—	—	—	30	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Sweden	1948	—	—	—	—	—	—	—	—	24	—	—	—	—	—	—	—	—	—	—	—	—	—
U.K.	1948	845	639	34	18	657	278	—	2	45	70	14	—	11	—	10	25	4	25	—	43	—	—
U.S.A.	1948	769	367	—	132	489†	52	—	1	6†	2†	578	105	94	75	1	596	128	52	—	47	—	—

* Bait.
† 1947.
‡ Not cod or related species.

tons (eviscerated, heads-off weight), compared with only 5,000 being used fresh. In 1947, out of a total of 229,000 tons allocated, 70,000 tons were dried and salted, 24,000 tons dried to produce 9,000 tons of stockfish and 40,000 tons used fresh or frozen. However, this year more drying and less freezing is reported qoth in Norway and Iceland.

Table 9. *Disposal of British herrings*

(thousand tons landed weight)

Treatment	Home consumption			Export			Total		
	1937	1948	1949	1937	1948	1949	1937	1948	1949
Fresh	40	50	47	—	—	—	77	82	64
Quick frozen	—	3	5	—	—	—			
'Klondyked'*	—	—	—	37	29	12			
Kippered	57	78	47	4	2	4	61	80	51
Bloatered	1	4	—	2	—	—	3	4	—
'Redded'	—	1	—	15	14	10	15	15	10
Canned	5	11	13	3	3	5	8	14	18
Salt-cured	—	3	1	121	49	35	121	52	36
Marinated	—	1	1	—	—	—	—	1	1
Meal and oil	1	18	22	2	—	—	3	18	22
TOTAL	104	169	136	184	97	66	288	266	202

* Mixed with ice and salt and despatched fresh in boxes on fast carrier vessels.

The trade in dried salted cod has not declined in this way and it is still exported from North Atlantic countries chiefly to the Mediterranean, South America and the Caribbean (see Table 10). But although the total quantity of cod salted has shown no downward trend, the proportion has decreased owing to the expansion in the cod fishing in northern waters between the wars, as is shown by Table 7.

Although related species, such as saithe and ling, are also salted, the proportion of the total landings so treated is much less than in the case of cod.

Table 8, which includes most of the industrially advanced countries with the notable exception of U.S.S.R., shows that, roughly speaking, quantities of about the same order are distributed in the 'fresh', i.e. unprocessed, state and dried, salted or smoked. The biggest single outlet, however, is meal and oil, if full Japanese production is included. Canning and freezing come well behind, accounting for perhaps about a million tons each or about 5% of the total.

Out of a total catch of herring and herring-like species of more than 3 million tons reported to F.A.O. by 20 countries in 1946 (Food and Agricultural Organization of the United Nations, 1948 *b*), only about 1,100,000 tons, or approximately one-third, was used for human consumption; about 1,100,000 tons went for meal and oil and 100,000 tons for bait, whilst about 700,000 was 'waste', including some

Table 10. *Approximate annual production and disposition of salt cod*, 1920–39

(thousands of tons)

		Producing country							Home production
		Newfoundland	Iceland	Norway	France	Canada	U.K.	Faroe Is.	
Total production	258·8	65·2	50·1	42·2	38·0	25·3	13·1	13·0	10·4
Importing country	Total imports								
Europe									
Spain	57·3	12·2	19·8	10·1	2·6	0·3	4·3	7·4	2·8
Portugal	39·8	10·9	6·3	15·3	1·5	0·2	3·3	1·2	4·9
Italy	38·5	7·3	14·3	1·9	6·6	2·3	0·8	4·2	
Greece	9·4	3·3	0·2	0·2	3·7	1·4	1·0	0·4	—
France	1·3	—	—	—	—	—	—	—	—
Total†	—	53%	98%	69%	—	12%	—	99%	—
S. America									
Brazil	26·8	11·7	0·1	0·5	0·2	1·7	7·6	—	—
Argentina	4·7	—	0·1	3·4	—	—	1·1	—	—
Total†	—	19%	2%	19%	—	10%	—	—	—
Caribbean									
Cuba	13·3	0·2	0·2	4·0	—	5·3	0·9	—	—
Dominican Republic	1·7	—	—	—	—	—	—	—	—
Total†	—	24%	1%	10%	—	55%	—	1%	—
N. America									
U.S.A.	19·4	5·4*	0·1	4·3	0·2	1·7	7·6	—	2·5
Total†	—	2%*	—	1%	—	23%	—	—	—
Others	19·7	2%†	—	1%†	—	—	—	—	—
TOTAL	231·7								

* Inconsistency reflecting conflicting data in Tables A–23 (p. 142) and A–36 (p. 157) of Gerhardsen & Gertenbach (1949) for exports of dried salt fish from Newfoundland to U.S.A. possibly produced by inclusion of Labrador with Newfoundland in some places.

† Percentage of country's production going to importing area.

small quantities used for fertilizer and for animal fodder other than meal. The marketed weight of various herring products in 16 out of the 20 countries was as follows: fresh and frozen, 297,000; salted, 382,000; dried only (Japan), 123,000; canned, 195,000; meal, 217,000; and oil, 104,000 tons.

The diverse character of the fish industries of various countries is also shown by Table 8.

The U.K., being small and compact, is able to distribute most of its fish, although drawn from as far away as Spitzbergen, in 'fresh' condition in ice. Most of the fish it catches is eaten by its own population. U.S.A. distributes only about half as much as we do in the fresh condition, while canning and fish-meal manufacture each account for about 50% more. Relatively small quantities are cured and frozen for internal distribution. However, freezing bulks much larger than in U.K. Over 100,000 tons of fresh, frozen and cured fish were imported before the war, and 20,000 tons of canned fish exported, rising to over 100,000 tons during the war.

Newfoundland relies almost entirely on its fish-salting industry. In Canada about equal proportions of the fish are allocated to freshing, freezing, salting and smoking, canning and reduction to fish-meal. Iceland, whose economy is based upon exports of fish, freshes large quantities aboard trawlers and carrier ships for export, freezes large quantities, salts rather less, and makes a good deal of fish-meal and oil. Norway freshes and salts a good deal, and freezes and cans rather less, but makes large quantities of meal and oil. Of a catch of herrings during the 1950 winter season of about 750,000 tons, just over 50,000 each went for freshing and salting and the remainder was allocated to meal and oil. Japan makes enormous quantities of fish-meal, cans a fair amount, salts a good deal (up to 200,000 tons before the war) and hard smokes salmon on a large scale (373,000 in 1943) as well as consuming quite a lot in the fresh state.

Fish-meal and oil production throughout the world was estimated at about 1,200,000 tons (Harrison, 1939), equivalent to 6–7 million tons landed weight, or a third of the world catch. A small proportion of this was made from the head, bones and adhering flesh of 'white' fish, after filleting, and also from surplus or 'condemned' white fish. These sources were the basis of the fish-meal industries of U.K. and Germany, as a by-product of their large, centralized trawling industries. Most of the meal, however, and about 150,000 tons of oil, was made from pelagic fatty fish. Japan, together with Korea, Formosa and Sakhalin, produced about 750,000 tons of meal, which was about two-thirds of the world output, and accounted for the major portion of their huge catch. This enormous quantity, the product of some 6,000 small plants, was apparently used almost entirely as a nitrogenous fertilizer, since the requirements of live-stock were negligible (Wood, 1948).

Recent F.A.O. statistics suggest that although the Japanese industry was at least until recently practically at a standstill, the production in the other major producing countries has recovered from the effects of the war and is still going up. These are relatively up-to-date, highly mechanized industries, that afford the chief, if not in some cases the only, outlet for the large-scale commercial fisheries which supply them with raw material. Probably only a small proportion of the oil is used for the production of edible fat, most being fed to animals or used in industry. Although most of the meal is used as animal-feeding stuff, a certain amount, e.g. menhaden, is used as fertilizer.

In Britain the manufacture of meal and oil from herrings, our chief pelagic fish, has hitherto been negligible. In 1948 some 2,500 tons of oil and 4,000 tons of meal were produced from surplus fish, but in 1949 nearly twice as much herring was allocated, over 40,000 tons, more than 10% of the catch. Although the Herring Industry Board's most recent target for the reduction of herrings in 1952 is some 140,000 tons, which is about the same as the present home consumption, it is not the intention to fish directly for the purpose of oil and meal manufacture.

In considering the figures in Table 8, it should be noted that there are wide fluctuations in production from year to year. Thus in the case of cod-liver oil, for example, in 1947, Norway reported 16,500 tons and Canada 4,000 tons, both more than double the 1948 figures.

PROBLEMS OF THE FISH INDUSTRY

It will be seen from the general picture of the world's fishing industry, outlined above, that there are two major technical problems, namely preservation and utilization, and one major biological, economic problem, namely ' overfishing '.

Dealing with the latter first, because it supplies the natural background, it can be seen from Table 7 that the amount of white fish which is now being taken out of the North Sea has decreased. This is considered by biologists to be due to the inroads on the stocks in a relatively small area made by the depredations of man's fishing operations. As a result, an ever-increasing intensity and efficiency of fishing effort is required to maintain the same weight of fish caught year after year and the average size of fish steadily decreases. After a rest during the war, when stocks increased, the same phenomenon has again put in an appearance. A similar tendency amongst halibut in the Pacific was apparently halted by an agreement between Canada and U.S.A. There is as yet little sign that fishing for pelagic species has reached its economic limit, except perhaps in the case of the Pacific pilchard, although there may be biological reasons to account for its recent decline.

In the case of the British trawling industry, larger vessels holding 200–300 tons of fish were built, mostly for Hull and Grimsby, to fish more distant waters, 1,500 miles or more away from our shores, where prolific fisheries for cod were developed in the Arctic. However, the length of the voyage home combined with the fishing-time required on the grounds to fill up economically (see Table 11) has outrun the preservative effect of gutting, washing and icing at sea. This has meant that little really fresh fish, less than six days old say, can be landed, and that a great deal of somewhat stale fish (more than 14 days old) is landed, which takes another day or two to be marketed and distributed throughout the country. Although not actually harmful, such fish is not particularly appetizing and tends to give fish generally rather a poor reputation. The limits suggested for the maximum period of freshness of iced fish are those obtained under ideal conditions of storage (Reay & Shewan, 1949). Under commercial conditions the 'life' is, if anything, shorter. Liquor is squeezed out under pressure in the fish-hold, resulting in losses of weight up to 15% (Cutting, in the press), which includes a proportion of the flavouring substances and some protein (Dyer & Dyer, 1947). Slight extensions of a few days in storage life can be effected by storing the iced fish in an atmosphere of carbon dioxide (Shewan, 1950), but there are technical problems here too. The only way in which fresher fish can be landed under existing conditions is to return sooner from the grounds, i.e. catch less fish per trip, which would be less economic owing to the high overheads involved in running large trawlers and the high proportion of steaming-time in the case of the distant grounds. The restriction of fishing practised in the 1930's to keep prices at an economic level, which has recently been resumed in view of the present fall-off in consumer demand, did actually result in better fish from shorter trips and better handling. Acute meat shortage has now led to unrestricted fishing again.

Table 11. *Steaming and round trip times for British trawlers*

Fishing-ground	a Average round trip 1948 and 1949 (days)	b Steaming time to grounds at 12 knots = age of freshest fish landed (days)	$a-b=$ Average age of stalest fish landed (days)	$\frac{\text{Steaming-time}}{\text{Fishing-time}}$ $\left(=\frac{2b}{a-2b}\right)$
North Sea	8	½–1*	7	0·23
Rockall (West of Scotland)	14	1–1½*	13	0·22
Faroes	17	2½*	14	0·42
Iceland	21	3½–4	17	0·56
Bear Island and Spitzbergen	23	4½	18	0·64
Barents Sea	25	5½–6	19	0·85
West of Greenland	27	7½–8½	19	1·46
Newfoundland	28	8	20	1·34

* 10 knots assumed, since mostly smaller and older vessels.

The only alternative is to freeze fish at sea whilst still perfectly fresh, when they can, if properly treated, be kept for months in perfect condition (Reay, Banks & Cutting, 1950). When a really satisfactory process of freezing at sea has been developed, the change-over would involve much capital expenditure on plant and equipment, and even when proved to be technically successful, would probably take some decades to develop fully in the industry.

The British pelagic fishery for herrings, which are mostly caught on overnight trips and often landed at outlying ports, has not yet been won over to the use of ice to preserve the fish at sea or its adequate use and conservation during subsequent transport and distribution (Reay & Shewan, unpublished). More could be done in these directions to improve the quality and area of penetration of our catch, which could probably be considerably increased if the market could be expanded. There is, however, the danger that vessels icing their fish would stay longer at sea, and even effectively insulated iced transport on land might only increase the area of penetration of somewhat stale herrings. Proper freezing and cold storage would permit distribution in perfect condition, as well as smoothing out the seasonal fluctuation in the catch, which is a feature of most pelagic fisheries, and thus provide first-quality fish all the year round (Banks, 1945).

Large export markets in the vast protein deficient areas of the world could also be penetrated by suitable preserved or dehydrated herrings (Cutting & Reay, 1944), provided that it could be made available at a cost that the populations could afford.

Far too high a proportion of the world catch of pelagic fish goes into industrial use, e.g. oil to produce soap or linoleum, and meal for use as fertilizer. These uses should be up-graded so that at least edible oils and animal-feeding meals are produced and preferably the fish used for direct human consumption (Reay & Cutting, in the press). At the same time, increased quantities of some of these species, particularly the North Sea herrings, could probably be caught if there were assured outlets for them. The rate of catching frequently exceeds the handling capacity at the port. If surpluses could be stored without offence, they could be converted into fish-meal and oil during the ‘ off ’ season. This is the object of a process recently developed at Torry Research Station in association with the Herring Industry Board, for which a patent has been applied. Herrings are stored in dilute alkali, which releases the oil and digests the protein, which then has to be recovered in some suitable form from the liquor (Herring Industry Board, 1949, 1950 ; Lovern, in preparation).

The apparent limit to the possible catch of fish and the phenomena of over-fishing make its adequate preservation and efficient utilization all the more essential. At the same, time only when suitable processes and markets exist will ‘ all-out ’ fishing of under-fished species be practicable.

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CONTRIBUTION TO THE DISCUSSION

Professor A. Wormall suggested that immunological investigations on fish tissues, including blood, might yield interesting and useful results. Little work appears to have been done in this field, though it is known that the protamines are not antigenic ; hence the absence, or relative infrequency, of undesirable immunological reactions following the injection of protamine-insulin complexes into diabetics.

Serological studies on animal, and to a lesser extent plant, proteins have yielded useful information about species differences amongst certain proteins, e.g. the caseinogens, serum globulins and thyroglobulins. It would be interesting to have similar data for fish proteins, for immunological methods often permit the demonstration of chemical differences which are not detectable, or detectable only with difficulty, by ordinary chemical methods.

Further work might possibly be done to find out whether there are blood-group factors in fish similar to those found in the blood of man and a few other animals. Professor Wormall stated that about 20 years ago, he failed to find any major blood-group differences amongst dogfish, but he suggested that a more complete examination of this subject might now be desirable in the light of the more modern knowledge about the rhesus and other blood factors.